

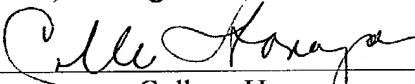
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Washington, D.C. 20231

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Colleen Hanagan

NATIONAL STAGE APPLICATION TRANSMITTAL LETTER
APPLICATION FILING UNDER 35 U.S.C. § 371

Transmitted herewith for filing is the patent application of:

Inventor(s)/Applicant(s):	Ruelle, Jean-Louis
International Application No.:	PCT/EP00/01468
International Published Appln. No.:	WO 00/52042
International Filing Date:	23 February 2000
Priority Filing Dates:	26 February 1999
Thirty Month Date:	26 August 2001
Title:	"IMMUNOGENIC COMPOUNDS"

1. **THIS NEW APPLICATION IS A NATIONAL STAGE APPLICATION UNDER PCT, CHAPTER II WITH A REQUEST FOR EXAMINATION WITHOUT DELAY TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US).**

- ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. § 371;
- ☐ This is a **SECOND** or subsequent submission of items concerning a filing under 35 U.S.C. § 371.

2. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).

3. A proper Demand for International Preliminary Examination was made by the 19TH month from the earliest claimed priority date.

4. Enclosed items are required for filing under 37 CFR § 1.53(b) and § 1.494(b) or § 1.495(b):

- ☒ One copy of International Publication No. WO 00/52042
- (a) ☒ is transmitted herewith (**required only if not transmitted by the International Bureau**)
- (b) ☐ has been transmitted by the International Bureau
- (c) ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)

Fees

☒ The basic national fee set forth in 37 CFR § 1.482 - International Preliminary Examination Fee not paid to USPTO but International Search Report prepared by the EPO or JPO - **\$860.00**

- ☒ Claims in Excess of 20 (2 @ \$18.00)
- ☐ Independent Claims in Excess of 3 (@ \$80.00)

5. Further enclosed are:

- ☒ One copy of International Preliminary Examination Report.
- ☒ One copy of International Search Report.
- ☐ One copy of Written Opinion.
- ☐ One copy of PCT Request as filed.
- ☐ One copy of Chapter II Demand as filed.

6. ☐ A translation of the International Application into English (35 U.S.C. § 371(c)(2))

7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. § 371(c)(3))

8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. § 371(c)(3))

9. Still additional papers enclosed:

- ☐ Assignment with Assignment Recordation Form Cover Sheet
- ☐ Verification Statement Claiming Small Entity Status
- ☐ Declaration or oath is enclosed executed by the inventor
- ☐ An Information Disclosure Statement under 37 CFR § 1.97 and § 1.98
- ☒ Return Acknowledgment Postcard

10. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
- A. Enclosed are:
- (a) ☐ Computer Readable Copy of the Sequence Listing
- (b) ☐ Paper Copy (identical to Computer Readable Copy) of the Sequence Listing
- B. ☐ Enclosed is a paper copy of the Sequence Listing. This paper copy and a Computer Readable Form thereof are identical with the Computer Readable Form in another application of the Applicant which is fully identified as follows:
- U.S. Application No.: @@
- Filed: @@
- Attorney Docket No.: @@
- which is believed to comply with the rules set forth in 37 CFR § 1.821 et. seq. Applicants requests pursuant to 37 CFR § 1.821(e) that this Computer Readable Form be used in the present application. **Please TRANSFER the sequence listing from the parent to this application.**
- C. ☐ Statement under 37 CFR § 1.821(f): **The information recorded in computer readable form is identical to the written Sequence Listing.**
- D. ☐ Statement under 37 CFR § 1.821(g) (required when Sequence Listing not submitted at the time of filing under 35 U.S.C. §111(a)) or 37 CFR §1.821(f) (required when Sequence Listing not submitted at the time of filing under the Patent Cooperation Treaty): **The submission of the Sequence Listing includes no new matter.**
- E. ☐ Amendment: Please enter the Sequence Listing into the application.

11. **Preliminary Amendment**

Prior to calculation of fees, kindly enter:

- ☒ Preliminary Amendment submitted herewith
- ☐ do not enter Preliminary Amendment

12. The correspondence address for this application is the Customer No. provided below:

Insert Bar Code Label Here:



25308

PATENT TRADEMARK OFFICE

13. Fee payment being made at this time is enclosed:

* Basic filing fee (\$860.00)	860.00
* Claims in Excess of 20	36.00
* (2 @ \$18.00)	
Independent Claims in Excess of 3	00.00
(@ \$80.00)	
* Total Fees enclosed:	<u>\$896.00</u>

14. The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Account No. 50-0258. This letter is filed in duplicate for accounting purposes.

Respectfully submitted,

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International Application No.: PCT/EP00/01468
Attorney Docket No.: BM45378

09/914168
513 Rec'd PCT/PTO 23 AUG 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: Ruelle
Docket No.: BM45378
Serial No.: Unknown
Filed: Herewith

Group Art Unit No.: Unknown
Examiner: Unknown

For: Immunogenic Compounds

PRELIMINARY AMENDMENT

Sir:

Applicant respectfully requests that this Preliminary Amendment be entered in this case before the calculation of fees and before examination of the subject application.

In the Claims:

Please delete the claims of the application as filed in the PCT and substitute therefor:

25. An isolated polypeptide comprising a member selected from the group consisting of
- (a) an amino acid sequence matching one of SEQ ID NOs:2 or 4;
 - (b) an immunogenic polypeptide comprising a fragment sequence of at least 15 amino acids that matches an aligned contiguous segment of one of SEQ ID NOs:2 or 4;

wherein the isolated polypeptide, when administered to a subject in a suitable composition which can include an adjuvant, or a suitable carrier coupled to the polypeptide, induces an antibody or T-cell immune response to a polypeptide having the sequence of one of SEQ ID NOs:2 or 4.

26. An isolated polynucleotide encoding a polypeptide of Claim 25 or the full complement to the isolated polynucleotide.

27. The isolated polypeptide of claim 25, wherein the polypeptide is according to (a).

28. An isolated polynucleotide encoding a polypeptide of Claim 27 or the full complement to the isolated polynucleotide.

29. The isolated polypeptide of claim 25, wherein the polypeptide is according to (b).

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30. An isolated polynucleotide encoding a polypeptide of Claim 29 or the full complement to the isolated polynucleotide.
31. The isolated polypeptide of claim 25, wherein the immunogenic fragment of (b) comprises at least 20 amino acids.
32. The isolated polypeptide of Claim 25 wherein the isolated polypeptide of (a) consists of one of SEQ ID NOs: 2 or 4.
33. An isolated polynucleotide encoding a polypeptide of Claim 32 or the full complement to the isolated polynucleotide.
34. A process for expressing the polynucleotide of Claim 33 comprising transforming a host cell with an expression vector comprising the polynucleotide and culturing the host cell under conditions sufficient for expression of the polynucleotide.
35. A fusion protein comprising the isolated polypeptide of Claim 25.
36. An isolated polynucleotide comprising the polynucleotide of one of SEQ ID NOs:1 or 3.
37. An isolated polynucleotide segment comprising a polynucleotide sequence or the full complement of the entire length of the polynucleotide sequence, wherein the polynucleotide sequence hybridizes to the full complement of one of SEQ ID NOs:1 or 3 minus the complement of any stop codon, wherein the hybridization conditions include incubation at 42 °C in a solution comprising: 50% formamide, 5x SSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 micrograms/ml denatured, sheared salmon sperm DNA, followed by washing in 0.1x SSC at 65°C; and, wherein the polynucleotide sequence is identical to one of SEQ ID NOs:1 or 3 minus any terminal stop codon, except that, over the entire length corresponding to one of SEQ ID NOs:1 or 3 minus any terminal stop codon, n_n nucleotides are substituted, inserted or deleted, wherein n_n satisfies the following expression

Claims

New claims 25-46 have been introduced. No new matter is added.

Support for the new claims is either apparent, or is as described in the text below. Support for compositions of the isolated polypeptide which include an adjuvant recited in the claims may be found, for example, at page 44, lines 14-15. Support for the hybridization conditions may be found, for example, at page 14, lines 21-25. Support for the recitation of sequence relatedness such as in claim 37 may be found in the specification, for example, at page 47, line 25 through page 48, line 20.

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Closing Remarks

Allowance of the pending claims is respectfully requested.

Respectfully submitted,



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IMMUNOGENIC COMPOUNDS

FIELD OF THE INVENTION

This invention relates to polynucleotides, (herein referred to as "BASB081 polynucleotide(s)"), polypeptides encoded by them (referred to herein as "BASB081" or "BASB081 polypeptide(s)"), recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including vaccines against bacterial infections. In a further aspect, the invention relates to diagnostic assays for detecting infection of certain pathogens.

BACKGROUND OF THE INVENTION

Moraxella catarrhalis (also named *Branhamella catarrhalis*) is a Gram negative bacteria frequently isolated from the human upper respiratory tract. It is responsible for several pathologies the main ones being otitis media in infants and children, and pneumonia in elderlies. It is also responsible of sinusitis, nosocomial infections and less frequently of invasive diseases.

Otitis media is an important childhood disease both by the number of cases and its potential sequelae. More than 3.5 millions cases are recorded every year in the United States, and it is estimated that 80 % of the children have experienced at least one episode of otitis before reaching the age of 3 (Klein, JO (1994) Clin.Inf.Dis 19:823). Left untreated, or becoming chronic, this disease may lead to hearing losses that could be temporary (in the case of fluid accumulation in the middle ear) or permanent (if the auditive nerve is damaged). In infants, such hearing losses may be responsible for a delayed speech learning.

Three bacterial species are primarily isolated from the middle ear of children with otitis media: *Streptococcus pneumoniae*, non typeable *Haemophilus influenza* (NTHi) and *M. catarrhalis*. They are present in 60 to 90 % of the cases. A review of recent studies shows that *S. pneumoniae* and NTHi represent both about 30 %, and *M. catarrhalis* about 15 % of

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considered as a virulence factor of the bacteria. An opsonizing activity has been observed in the sera of children recovering from otitis media.

The antigens targetted by these different immune responses in humans have not been identified, with the exception of OMP B1, a 84 kDa protein which expression is regulated by iron, and that is recognized by the sera of patients with pneumonia (Sethi, S, et al. (1995) Infect.Immun. 63:1516) , and of UspA1 and UspA2 (Chen D. et al.(1999), Infect.Immun. 67:1310).

A few other membrane proteins present on the surface of *M. catarrhalis* have been characterized using biochemical method, or for their potential implication in the induction of a protective immunity (for review, see Murphy, TF (1996) Microbiol.Rev. 60:267). In a mouse pneumonia model, the presence of antibodies raised against some of them (UspA, CopB) favors a faster clearance of the pulmonary infection. Another polypeptide (OMP CD) is highly conserved among *M. catarrhalis* strains, and presents homologies with a porin of *Pseudomonas aeruginosa*, which has been demonstrated efficacious against this bacterium in animal models.

The frequency of *Moraxella catarrhalis* infections has risen dramatically in the past few decades. This has been attributed to the emergence of multiply antibiotic resistant strains and an increasing population of people with weakened immune systems. It is no longer uncommon to isolate *Moraxella catarrhalis* strains that are resistant to some or all of the standard antibiotics. This phenomenon has created an unmet medical need and demand for new anti-microbial agents, vaccines, drug screening methods, and diagnostic tests for this organism.

SUMMARY OF THE INVENTION

The present invention relates to BASB081, in particular BASB081 polypeptides and BASB081 polynucleotides, recombinant materials and methods for their production. In another aspect, the invention relates to methods for using such polypeptides and polynucleotides, including prevention and treatment of microbial diseases, amongst others. In a further aspect, the invention relates to diagnostic assays for detecting diseases associated with microbial infections and conditions associated with such infections, such as assays for detecting expression or activity of BASB081 polynucleotides or polypeptides.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

DESCRIPTION OF THE INVENTION

The invention relates to BASB081 polypeptides and polynucleotides as described in greater detail below. In particular, the invention relates to polypeptides and polynucleotides of BASB081 of *Moraxella catarrhalis*, which is related by amino acid sequence homology to *Neisseria meningitidis* omp85 outer membrane protein. The invention relates especially to BASB081 having the nucleotide and amino acid sequences set out in SEQ ID NO:1 or 3 and SEQ ID NO:2 or 4 respectively. It is understood that sequences recited in the Sequence Listing below as "DNA" represent an exemplification of one embodiment of the invention, since those of ordinary skill will recognize that such sequences can be usefully employed in polynucleotides in general, including ribopolynucleotides.

Polypeptides

In one aspect of the invention there are provided polypeptides of *Moraxella catarrhalis* referred to herein as "BASB081" and "BASB081 polypeptides" as well as biologically, diagnostically, prophylactically, clinically or therapeutically useful variants thereof, and compositions comprising the same.

The present invention further provides for:

- (a) an isolated polypeptide which comprises an amino acid sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97-99% or exact identity, to that of SEQ ID NO:2 or 4;
- (b) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:1 or 3 respectively; or
- (c) a polypeptide encoded by an isolated polynucleotide comprising a polynucleotide sequence encoding a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97-99% or exact identity, to the amino acid sequence of SEQ ID NO:2 or 4.

The BASB081 polypeptides provided in SEQ ID NO:2 or 4 are the BASB081 polypeptides from *Moraxella catarrhalis* strain Mc2931 (ATCC 43617).

The invention also provides an immunogenic fragment of a BASB081 polypeptide, that is, a contiguous portion of the BASB081 polypeptide which has the same or substantially the same immunogenic activity as the polypeptide comprising the amino acid sequence of SEQ ID NO:2 or 4; That is to say, the fragment (if necessary when coupled to a carrier) is capable of raising an immune response which recognises the BASB081 polypeptide. Such an immunogenic fragment may include, for example, the BASB081 polypeptide lacking an N-terminal leader sequence, and/or a transmembrane domain and/or a C-terminal anchor domain. In a preferred aspect the immunogenic fragment of BASB081 according to the invention comprises substantially all of the extracellular domain of a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, most preferably at least 97-99% identity, to that of SEQ ID NO:2 or 4 over the entire length of SEQ ID NO:2

Particularly preferred are variants in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acids are substituted, deleted, or added in any combination.

The polypeptides, or immunogenic fragments, of the invention may be in the form of the "mature" protein or may be a part of a larger protein such as a precursor or a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, or an additional sequence for stability during recombinant production. Furthermore, addition of exogenous polypeptide or lipid tail or polynucleotide sequences to increase the immunogenic potential of the final molecule is also considered.

In one aspect, the invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa.

Furthermore, this invention relates to processes for the preparation of these fusion proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

The proteins may be chemically conjugated, or expressed as recombinant fusion proteins allowing increased levels to be produced in an expression system as compared to non-fused protein. The fusion partner may assist in providing T helper epitopes

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Another aspect of the invention relates to isolated polynucleotides, including at least one full length gene, that encodes a BASB081 polypeptide having a deduced amino acid sequence of SEQ ID NO:2 or 4 and polynucleotides closely related thereto and variants thereof.

In another particularly preferred embodiment of the invention there is a BASB081 polypeptide from *Moraxella catarrhalis* comprising or consisting of an amino acid sequence of SEQ ID NO:2 or 4 or a variant thereof.

Using the information provided herein, such as a polynucleotide sequence set out in SEQ ID NO:1 or 3, a polynucleotide of the invention encoding BASB081 polypeptide may be obtained using standard cloning and screening methods, such as those for cloning and sequencing chromosomal DNA fragments from bacteria using *Moraxella catarrhalis* Catlin cells as starting material, followed by obtaining a full length clone. For example, to obtain a polynucleotide sequence of the invention, such as a polynucleotide sequence given in SEQ ID NO:1 or 3, typically a library of clones of chromosomal DNA of *Moraxella catarrhalis* Catlin in *E.coli* or some other suitable host is probed with a radiolabeled oligonucleotide, preferably a 17-mer or longer, derived from a partial sequence. Clones carrying DNA identical to that of the probe can then be distinguished using stringent hybridization conditions. By sequencing the individual clones thus identified by hybridization with sequencing primers designed from the original polypeptide or polynucleotide sequence it is then possible to extend the polynucleotide sequence in both directions to determine a full length gene sequence. Conveniently, such sequencing is performed, for example, using denatured double stranded DNA prepared from a plasmid clone. Suitable techniques are described by Maniatis, T., Fritsch, E.F. and Sambrook et al., *MOLECULAR CLONING, A LABORATORY MANUAL*, 2nd Ed.; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). (see in particular Screening By Hybridization 1.90 and Sequencing Denatured Double-Stranded DNA Templates 13.70). Direct genomic DNA sequencing may also be performed to obtain a full length gene

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sequence. Illustrative of the invention, each polynucleotide set out in SEQ ID NO:1 or 3 was discovered in a DNA library derived from *Moraxella catarrhalis*.

Moreover, each DNA sequence set out in SEQ ID NO:1 or 3 contains an open reading frame encoding a protein having about the number of amino acid residues set forth in SEQ ID NO:2 or 4 with a deduced molecular weight that can be calculated using amino acid residue molecular weight values well known to those skilled in the art.

The polynucleotide of SEQ ID NO:1, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2758 of SEQ ID NO:1, encodes the polypeptide of SEQ ID NO:2.

The polynucleotide of SEQ ID NO:3, between the start codon at nucleotide number 1 and the stop codon which begins at nucleotide number 2668 of SEQ ID NO:3, encodes the polypeptide of SEQ ID NO:4.

In a further aspect, the present invention provides for an isolated polynucleotide comprising or consisting of:

- (a) a polynucleotide sequence which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97-99% or exact identity to SEQ ID NO:1 or 3 over the entire length of SEQ ID NO:1 or 3 respectively; or
- (b) a polynucleotide sequence encoding a polypeptide which has at least 85% identity, preferably at least 90% identity, more preferably at least 95% identity, even more preferably at least 97-99% or 100% exact, to the amino acid sequence of SEQ ID NO:2 or 4, over the entire length of SEQ ID NO:2 or 4 respectively.

A polynucleotide encoding a polypeptide of the present invention, including homologs and orthologs from species other than *Moraxella catarrhalis*, may be obtained by a process which comprises the steps of screening an appropriate library under stringent hybridization

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redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2 or 4.

The term "polynucleotide encoding a polypeptide" as used herein encompasses polynucleotides that include a sequence encoding a polypeptide of the invention, particularly a bacterial polypeptide and more particularly a polypeptide of the *Moraxella catarrhalis* BASB081 having an amino acid sequence set out in SEQ ID NO:2 or 4. The term also encompasses polynucleotides that include a single continuous region or discontinuous regions encoding the polypeptide (for example, polynucleotides interrupted by integrated phage, an integrated insertion sequence, an integrated vector sequence, an integrated transposon sequence, or due to RNA editing or genomic DNA reorganization) together with additional regions, that also may contain coding and/or non-coding sequences.

The invention further relates to variants of the polynucleotides described herein that encode variants of a polypeptide having a deduced amino acid sequence of SEQ ID NO:2 or 4. Fragments of polynucleotides of the invention may be used, for example, to synthesize full-length polynucleotides of the invention.

Further particularly preferred embodiments are polynucleotides encoding BASB081 variants, that have the amino acid sequence of BASB081 polypeptide of SEQ ID NO:2 or 4 in which several, a few, 5 to 10, 1 to 5, 1 to 3, 2, 1 or no amino acid residues are substituted, modified, deleted and/or added, in any combination. Especially preferred among these are silent substitutions, additions and deletions, that do not alter the properties and activities of BASB081 polypeptide.

Further preferred embodiments of the invention are polynucleotides that are at least 85% identical over their entire length to a polynucleotide encoding BASB081 polypeptide having an amino acid sequence set out in SEQ ID NO:2 or 4, and polynucleotides that are complementary to such polynucleotides. Alternatively, most highly preferred are

The invention also provides a polynucleotide consisting of or comprising a polynucleotide sequence obtained by screening an appropriate library containing the complete gene for a polynucleotide sequence set forth in SEQ ID NO:1 or 3 under stringent hybridization conditions with a probe having the sequence of said polynucleotide sequence set forth in SEQ ID NO:1 or 3 or a fragment thereof; and isolating said polynucleotide sequence. Fragments useful for obtaining such a polynucleotide include, for example, probes and primers fully described elsewhere herein.

As discussed elsewhere herein regarding polynucleotide assays of the invention, for instance, the polynucleotides of the invention, may be used as a hybridization probe for RNA, cDNA and genomic DNA to isolate full-length cDNAs and genomic clones encoding BASB081 and to isolate cDNA and genomic clones of other genes that have a high identity, particularly high sequence identity, to the BASB081 gene. Such probes generally will comprise at least 15 nucleotide residues or base pairs. Preferably, such probes will have at least 30 nucleotide residues or base pairs and may have at least 50 nucleotide residues or base pairs. Particularly preferred probes will have at least 20 nucleotide residues or base pairs and will have less than 30 nucleotide residues or base pairs.

A coding region of a BASB081 gene may be isolated by screening using a DNA sequence provided in SEQ ID NO:1 or 3 to synthesize an oligonucleotide probe. A labeled oligonucleotide having a sequence complementary to that of a gene of the invention is then used to screen a library of cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

There are several methods available and well known to those skilled in the art to obtain full-length DNAs, or extend short DNAs, for example those based on the method of Rapid Amplification of cDNA ends (RACE) (see, for example, Frohman, *et al.*, *PNAS USA* 85: 8998-9002, 1988). Recent modifications of the technique, exemplified by the Marathon™

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technology (Clontech Laboratories Inc.) for example, have significantly simplified the search for longer cDNAs. In the Marathon™ technology, cDNAs have been prepared from mRNA extracted from a chosen tissue and an 'adaptor' sequence ligated onto each end. Nucleic acid amplification (PCR) is then carried out to amplify the "missing" 5' end of the DNA using a combination of gene specific and adaptor specific oligonucleotide primers. The PCR reaction is then repeated using "nested" primers, that is, primers designed to anneal within the amplified product (typically an adaptor specific primer that anneals further 3' in the adaptor sequence and a gene specific primer that anneals further 5' in the selected gene sequence). The products of this reaction can then be analyzed by DNA sequencing and a full-length DNA constructed either by joining the product directly to the existing DNA to give a complete sequence, or carrying out a separate full-length PCR using the new sequence information for the design of the 5' primer.

The polynucleotides and polypeptides of the invention may be employed, for example, as research reagents and materials for discovery of treatments of and diagnostics for diseases, particularly human diseases, as further discussed herein relating to polynucleotide assays.

The polynucleotides of the invention that are oligonucleotides derived from a sequence of SEQ ID NOS:1 or 3 may be used in the processes herein as described, but preferably for PCR, to determine whether or not the polynucleotides identified herein in whole or in part are transcribed in bacteria in infected tissue. It is recognized that such sequences will also have utility in diagnosis of the stage of infection and type of infection the pathogen has attained.

The invention also provides polynucleotides that encode a polypeptide that is the mature protein plus additional amino or carboxyl-terminal amino acids, or amino acids interior to the mature polypeptide (when the mature form has more than one polypeptide chain, for instance). Such sequences may play a role in processing of a protein from precursor to a mature form, may allow protein transport, may lengthen or shorten protein half-life or may

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facilitate manipulation of a protein for assay or production, among other things. As generally is the case *in vivo*, the additional amino acids may be processed away from the mature protein by cellular enzymes.

For each and every polynucleotide of the invention there is provided a polynucleotide complementary to it. It is preferred that these complementary polynucleotides are fully complementary to each polynucleotide with which they are complementary.

A precursor protein, having a mature form of the polypeptide fused to one or more prosequences may be an inactive form of the polypeptide. When prosequences are removed such inactive precursors generally are activated. Some or all of the prosequences may be removed before activation. Generally, such precursors are called proproteins.

In addition to the standard A, G, C, T/U representations for nucleotides, the term "N" may also be used in describing certain polynucleotides of the invention. "N" means that any of the four DNA or RNA nucleotides may appear at such a designated position in the DNA or RNA sequence, except it is preferred that N is not a nucleic acid that when taken in combination with adjacent nucleotide positions, when read in the correct reading frame, would have the effect of generating a premature termination codon in such reading frame.

In sum, a polynucleotide of the invention may encode a mature protein, a mature protein plus a leader sequence (which may be referred to as a preprotein), a precursor of a mature protein having one or more prosequences that are not the leader sequences of a preprotein, or a preproprotein, which is a precursor to a proprotein, having a leader sequence and one or more prosequences, which generally are removed during processing steps that produce active and mature forms of the polypeptide.

polynucleotides and/or to express a polypeptide in a host may be used for expression in this regard. The appropriate DNA sequence may be inserted into the expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook *et al.*, *MOLECULAR CLONING, A LABORATORY MANUAL*, (*supra*).

In recombinant expression systems in eukaryotes, for secretion of a translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals.

Polypeptides of the present invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, ion metal affinity chromatography (IMAC) is employed for purification. Well known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during intracellular synthesis, isolation and or purification.

The expression system may also be a recombinant live microorganism, such as a virus or bacterium. The gene of interest can be inserted into the genome of a live recombinant virus or bacterium. Inoculation and *in vivo* infection with this live vector will lead to *in vivo* expression of the antigen and induction of immune responses. Viruses and bacteria used for this purpose are for instance: poxviruses (e.g; vaccinia, fowlpox, canarypox), alphaviruses (Sindbis virus, Semliki Forest Virus, Venezuelan Equine Encephalitis Virus), adenoviruses, adeno-associated virus, picornaviruses (poliovirus, rhinovirus), herpesviruses (varicella zoster virus, etc), Listeria, Salmonella, Shigella, BCG. These

polynucleotide fragments in gels as compared to a reference sequence. This may be carried out with or without denaturing agents. Polynucleotide differences may also be detected by direct DNA or RNA sequencing. See, for example, Myers *et al.*, *Science*, 230: 1242 (1985). Sequence changes at specific locations also may be revealed by nuclease protection assays, such as RNase, V1 and S1 protection assay or a chemical cleavage method. See, for example, Cotton *et al.*, *Proc. Natl. Acad. Sci., USA*, 85: 4397-4401 (1985).

In another embodiment, an array of oligonucleotide probes comprising BASB081 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of, for example, genetic mutations, serotype, taxonomic classification or identification. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability (see, for example, Chee *et al.*, *Science*, 274: 610 (1996)).

Thus in another aspect, the present invention relates to a diagnostic kit which comprises:

- (a) a polynucleotide of the present invention, preferably the nucleotide sequence of SEQ ID NO:1 or 3, or a fragment thereof ;
- (b) a nucleotide sequence complementary to that of (a);
- (c) a polypeptide of the present invention, preferably the polypeptide of SEQ ID NO:2 or 4 or a fragment thereof; or
- (d) an antibody to a polypeptide of the present invention, preferably to the polypeptide of SEQ ID NO:2 or 4.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component. Such a kit will be of use in diagnosing a disease or susceptibility to a Disease, among others.

This invention also relates to the use of polynucleotides of the present invention as diagnostic reagents. Detection of a mutated form of a polynucleotide of the invention,

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preferably SEQ ID NO:1 or 3, which is associated with a disease or pathogenicity will provide a diagnostic tool that can add to, or define, a diagnosis of a disease, a prognosis of a course of disease, a determination of a stage of disease, or a susceptibility to a disease, which results from under-expression, over-expression or altered expression of the polynucleotide. Organisms, particularly infectious organisms, carrying mutations in such polynucleotide may be detected at the polynucleotide level by a variety of techniques, such as those described elsewhere herein.

Cells from an organism carrying mutations or polymorphisms (allelic variations) in a polynucleotide and/or polypeptide of the invention may also be detected at the polynucleotide or polypeptide level by a variety of techniques, to allow for serotyping, for example. For example, RT-PCR can be used to detect mutations in the RNA. It is particularly preferred to use RT-PCR in conjunction with automated detection systems, such as, for example, GeneScan. RNA, cDNA or genomic DNA may also be used for the same purpose, PCR. As an example, PCR primers complementary to a polynucleotide encoding BASB081 polypeptide can be used to identify and analyze mutations.

The invention further provides primers with 1, 2, 3 or 4 nucleotides removed from the 5' and/or the 3' end. These primers may be used for, among other things, amplifying BASB081 DNA and/or RNA isolated from a sample derived from an individual, such as a bodily material. The primers may be used to amplify a polynucleotide isolated from an infected individual, such that the polynucleotide may then be subject to various techniques for elucidation of the polynucleotide sequence. In this way, mutations in the polynucleotide sequence may be detected and used to diagnose and/or prognose the infection or its stage or course, or to serotype and/or classify the infectious agent.

The invention further provides a process for diagnosing, disease, preferably bacterial infections, more preferably infections caused by *Moraxella catarrhalis*, comprising determining from a sample derived from an individual, such as a bodily material, an

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increased level of expression of polynucleotide having a sequence of SEQ ID NO:1 or 3. Increased or decreased expression of a BASB081 polynucleotide can be measured using any one of the methods well known in the art for the quantitation of polynucleotides, such as, for example, amplification, PCR, RT-PCR, RNase protection, Northern blotting, spectrometry and other hybridization methods.

In addition, a diagnostic assay in accordance with the invention for detecting over-expression of BASB081 polypeptide compared to normal control tissue samples may be used to detect the presence of an infection, for example. Assay techniques that can be used to determine levels of a BASB081 polypeptide, in a sample derived from a host, such as a bodily material, are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis, antibody sandwich assays, antibody detection and ELISA assays.

The polynucleotides of the invention may be used as components of polynucleotide arrays, preferably high density arrays or grids. These high density arrays are particularly useful for diagnostic and prognostic purposes. For example, a set of spots each comprising a different gene, and further comprising a polynucleotide or polynucleotides of the invention, may be used for probing, such as using hybridization or nucleic acid amplification, using a probes obtained or derived from a bodily sample, to determine the presence of a particular polynucleotide sequence or related sequence in an individual. Such a presence may indicate the presence of a pathogen, particularly *Moraxella catarrhalis*, and may be useful in diagnosing and/or prognosing disease or a course of disease. A grid comprising a number of variants of the polynucleotide sequence of SEQ ID NO:1 or 3 are preferred. Also preferred is a comprising a number of variants of a polynucleotide sequence encoding the polypeptide sequence of SEQ ID NO:2 or 4.

Antibodies

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The polypeptides and polynucleotides of the invention or variants thereof, or cells expressing the same can be used as immunogens to produce antibodies immunospecific for such polypeptides or polynucleotides respectively.

In certain preferred embodiments of the invention there are provided antibodies against BASB081 polypeptides or polynucleotides.

Antibodies generated against the polypeptides or polynucleotides of the invention can be obtained by administering the polypeptides and/or polynucleotides of the invention, or epitope-bearing fragments of either or both, analogues of either or both, or cells expressing either or both, to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique known in the art that provides antibodies produced by continuous cell line cultures can be used. Examples include various techniques, such as those in Kohler, G. and Milstein, C., *Nature* 256: 495-497 (1975); Kozbor *et al.*, *Immunology Today* 4: 72 (1983); Cole *et al.*, pg. 77-96 in *MONOCLONAL ANTIBODIES AND CANCER THERAPY*, Alan R. Liss, Inc. (1985).

Techniques for the production of single chain antibodies (U.S. Patent No. 4,946,778) can be adapted to produce single chain antibodies to polypeptides or polynucleotides of this invention. Also, transgenic mice, or other organisms or animals, such as other mammals, may be used to express humanized antibodies immunospecific to the polypeptides or polynucleotides of the invention.

Alternatively, phage display technology may be utilized to select antibody genes with binding activities towards a polypeptide of the invention either from repertoires of PCR amplified v-genes of lymphocytes from humans screened for possessing anti-BASB081 or from naive libraries (McCafferty, *et al.*, (1990), *Nature* 348, 552-554; Marks, *et al.*, (1992) *Biotechnology* 10, 779-783). The affinity of these antibodies can also be improved by, for example, chain shuffling (Clackson *et al.*, (1991) *Nature* 352: 628).

indirectly associated with the candidate compound. Alternatively, the screening method may involve competition with a labeled competitor. Further, these screening methods may test whether the candidate compound results in a signal generated by activation or inhibition of the polypeptide or polynucleotide, using detection systems appropriate to the cells comprising the polypeptide or polynucleotide. Inhibitors of activation are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed. Constitutively active polypeptide and/or constitutively expressed polypeptides and polynucleotides may be employed in screening methods for inverse agonists or inhibitors, in the absence of an agonist or inhibitor, by testing whether the candidate compound results in inhibition of activation of the polypeptide or polynucleotide, as the case may be. Further, the screening methods may simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide or polynucleotide of the present invention, to form a mixture, measuring BASB081 polypeptide and/or polynucleotide activity in the mixture, and comparing the BASB081 polypeptide and/or polynucleotide activity of the mixture to a standard. Fusion proteins, such as those made from Fc portion and BASB081 polypeptide, as hereinbefore described, can also be used for high-throughput screening assays to identify antagonists of the polypeptide of the present invention, as well as of phylogenetically and and/or functionally related polypeptides (see D. Bennett *et al.*, J Mol Recognition, 8:52-58 (1995); and K. Johanson *et al.*, J Biol Chem, 270(16):9459-9471 (1995)).

The polynucleotides, polypeptides and antibodies that bind to and/or interact with a polypeptide of the present invention may also be used to configure screening methods for detecting the effect of added compounds on the production of mRNA and/or polypeptide in cells. For example, an ELISA assay may be constructed for measuring secreted or cell associated levels of polypeptide using monoclonal and polyclonal antibodies by standard methods known in the art. This can be used to discover agents which may inhibit or

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enhance the production of polypeptide (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues.

The invention also provides a method of screening compounds to identify those which enhance (agonist) or block (antagonist) the action of BASB081 polypeptides or polynucleotides, particularly those compounds that are bacteriostatic and/or bactericidal. The method of screening may involve high-throughput techniques. For example, to screen for agonists or antagonists, a synthetic reaction mix, a cellular compartment, such as a membrane, cell envelope or cell wall, or a preparation of any thereof, comprising BASB081 polypeptide and a labeled substrate or ligand of such polypeptide is incubated in the absence or the presence of a candidate molecule that may be a BASB081 agonist or antagonist. The ability of the candidate molecule to agonize or antagonize the BASB081 polypeptide is reflected in decreased binding of the labeled ligand or decreased production of product from such substrate. Molecules that bind gratuitously, *i.e.*, without inducing the effects of BASB081 polypeptide are most likely to be good antagonists. Molecules that bind well and, as the case may be, increase the rate of product production from substrate, increase signal transduction, or increase chemical channel activity are agonists. Detection of the rate or level of, as the case may be, production of product from substrate, signal transduction, or chemical channel activity may be enhanced by using a reporter system. Reporter systems that may be useful in this regard include but are not limited to colorimetric, labeled substrate converted into product, a reporter gene that is responsive to changes in BASB081 polynucleotide or polypeptide activity, and binding assays known in the art.

Another example of an assay for BASB081 agonists is a competitive assay that combines BASB081 and a potential agonist with BASB081-binding molecules, recombinant BASB081 binding molecules, natural substrates or ligands, or substrate or ligand mimetics, under appropriate conditions for a competitive inhibition assay. BASB081 can be labeled, such as by radioactivity or a colorimetric compound, such that the number of BASB081

molecules bound to a binding molecule or converted to product can be determined accurately to assess the effectiveness of the potential antagonist.

Potential antagonists include, among others, small organic molecules, peptides, polypeptides and antibodies that bind to a polynucleotide and/or polypeptide of the invention and thereby inhibit or extinguish its activity or expression. Potential antagonists also may be small organic molecules, a peptide, a polypeptide such as a closely related protein or antibody that binds the same sites on a binding molecule, such as a binding molecule, without inducing BASB081-induced activities, thereby preventing the action or expression of BASB081 polypeptides and/or polynucleotides by excluding BASB081 polypeptides and/or polynucleotides from binding.

Potential antagonists include a small molecule that binds to and occupies the binding site of the polypeptide thereby preventing binding to cellular binding molecules, such that normal biological activity is prevented. Examples of small molecules include but are not limited to small organic molecules, peptides or peptide-like molecules. Other potential antagonists include antisense molecules (see Okano, *J. Neurochem.* 56: 560 (1991); *OLIGODEOXYNUCLEOTIDES AS ANTISENSE INHIBITORS OF GENE EXPRESSION*, CRC Press, Boca Raton, FL (1988), for a description of these molecules). Preferred potential antagonists include compounds related to and variants of BASB081.

In a further aspect, the present invention relates to genetically engineered soluble fusion proteins comprising a polypeptide of the present invention, or a fragment thereof, and various portions of the constant regions of heavy or light chains of immunoglobulins of various subclasses (IgG, IgM, IgA, IgE). Preferred as an immunoglobulin is the constant part of the heavy chain of human IgG, particularly IgG1, where fusion takes place at the hinge region. In a particular embodiment, the Fc part can be removed simply by incorporation of a cleavage sequence which can be cleaved with blood clotting factor Xa. Furthermore, this invention relates to processes for the preparation of these fusion

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proteins by genetic engineering, and to the use thereof for drug screening, diagnosis and therapy. A further aspect of the invention also relates to polynucleotides encoding such fusion proteins. Examples of fusion protein technology can be found in International Patent Application Nos. WO94/29458 and WO94/22914.

Each of the polynucleotide sequences provided herein may be used in the discovery and development of antibacterial compounds. The encoded protein, upon expression, can be used as a target for the screening of antibacterial drugs. Additionally, the polynucleotide sequences encoding the amino terminal regions of the encoded protein or Shine-Delgarno or other translation facilitating sequences of the respective mRNA can be used to construct antisense sequences to control the expression of the coding sequence of interest.

The invention also provides the use of the polypeptide, polynucleotide, agonist or antagonist of the invention to interfere with the initial physical interaction between a pathogen or pathogens and a eukaryotic, preferably mammalian, host responsible for sequelae of infection. In particular, the molecules of the invention may be used: in the prevention of adhesion of bacteria, in particular gram positive and/or gram negative bacteria, to eukaryotic, preferably mammalian, extracellular matrix proteins on in-dwelling devices or to extracellular matrix proteins in wounds; to block bacterial adhesion between eukaryotic, preferably mammalian, extracellular matrix proteins and bacterial BASB081 proteins that mediate tissue damage and/or; to block the normal progression of pathogenesis in infections initiated other than by the implantation of in-dwelling devices or by other surgical techniques.

In accordance with yet another aspect of the invention, there are provided BASB081 agonists and antagonists, preferably bacteristatic or bactericidal agonists and antagonists.

The antagonists and agonists of the invention may be employed, for instance, to prevent, inhibit and/or treat diseases.

In a further aspect, the present invention relates to mimotopes of the polypeptide of the invention. A mimotope is a peptide sequence, sufficiently similar to the native peptide (sequentially or structurally), which is capable of being recognised by antibodies which recognise the native peptide; or is capable of raising antibodies which recognise the native peptide when coupled to a suitable carrier.

Peptide mimotopes may be designed for a particular purpose by addition, deletion or substitution of elected amino acids. Thus, the peptides may be modified for the purposes of ease of conjugation to a protein carrier. For example, it may be desirable for some chemical conjugation methods to include a terminal cysteine. In addition it may be desirable for peptides conjugated to a protein carrier to include a hydrophobic terminus distal from the conjugated terminus of the peptide, such that the free unconjugated end of the peptide remains associated with the surface of the carrier protein. Thereby presenting the peptide in a conformation which most closely resembles that of the peptide as found in the context of the whole native molecule. For example, the peptides may be altered to have an N-terminal cysteine and a C-terminal hydrophobic amidated tail. Alternatively, the addition or substitution of a D-stereoisomer form of one or more of the amino acids may be performed to create a beneficial derivative, for example to enhance stability of the peptide.

Alternatively, peptide mimotopes may be identified using antibodies which are capable themselves of binding to the polypeptides of the present invention using techniques such as phage display technology (EP 0 552 267 B1). This technique, generates a large number of peptide sequences which mimic the structure of the native peptides and are, therefore, capable of binding to anti-native peptide antibodies, but may not necessarily themselves share significant sequence homology to the native polypeptide.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in an individual, particularly a mammal, preferably humans, which comprises inoculating the individual with BASB081 polynucleotide and/or polypeptide, or a fragment or variant thereof, adequate to produce antibody and/ or T cell immune response to protect said individual from infection, particularly bacterial infection and most particularly *Moraxella catarrhalis* infection. Also provided are methods whereby such immunological response slows bacterial replication. Yet another aspect of the invention relates to a method of inducing immunological response in an individual which comprises delivering to such individual a nucleic acid vector, sequence or ribozyme to direct expression of BASB081 polynucleotide and/or polypeptide, or a fragment or a variant thereof, for expressing BASB081 polynucleotide and/or polypeptide, or a fragment or a variant thereof *in vivo* in order to induce an immunological response, such as, to produce antibody and/ or T cell immune response, including, for example, cytokine-producing T cells or cytotoxic T cells, to protect said individual, preferably a human, from disease, whether that disease is already established within the individual or not. One example of administering the gene is by accelerating it into the desired cells as a coating on particles or otherwise. Such nucleic acid vector may comprise DNA, RNA, a ribozyme, a modified nucleic acid, a DNA/RNA hybrid, a DNA-protein complex or an RNA-protein complex.

A further aspect of the invention relates to an immunological composition that when introduced into an individual, preferably a human, capable of having induced within it an immunological response, induces an immunological response in such individual to a BASB081 polynucleotide and/or polypeptide encoded therefrom, wherein the composition comprises a recombinant BASB081 polynucleotide and/or polypeptide encoded therefrom and/or comprises DNA and/or RNA which encodes and expresses an antigen of said BASB081 polynucleotide, polypeptide encoded therefrom, or other polypeptide of the invention. The immunological response may be used therapeutically or prophylactically and may take the form of antibody immunity and/or cellular immunity, such as cellular immunity arising from CTL or CD4+ T cells.

A BASB081 polypeptide or a fragment thereof may be fused with co-protein or chemical moiety which may or may not by itself produce antibodies, but which is capable of stabilizing the first protein and producing a fused or modified protein which will have antigenic and/or immunogenic properties, and preferably protective properties. Thus fused recombinant protein, preferably further comprises an antigenic co-protein, such as lipoprotein D from *Haemophilus influenzae*, Glutathione-S-transferase (GST) or beta-galactosidase, or any other relatively large co-protein which solubilizes the protein and facilitates production and purification thereof. Moreover, the co-protein may act as an adjuvant in the sense of providing a generalized stimulation of the immune system of the organism receiving the protein. The co-protein may be attached to either the amino- or carboxy-terminus of the first protein.

In a vaccine composition according to the invention, a BASB081 polypeptide and/or polynucleotide, or a fragment, or a mimotope, or a variant thereof may be present in a vector, such as the live recombinant vectors described above for example live bacterial vectors.

Also suitable are non-live vectors for the BASB081 polypeptide, for example bacterial outer-membrane vesicles or "blebs". OM blebs are derived from the outer membrane of the two-layer membrane of Gram-negative bacteria and have been documented in many Gram-negative bacteria (Zhou, L. *et al.* 1998. *FEMS Microbiol. Lett.* 163:223-228) including *C. trachomatis* and *C. psittaci*. A non-exhaustive list of bacterial pathogens reported to produce blebs also includes: *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella melitensis*, *Brucella ovis*, *Escherichia coli*, *Haemophilus influenza*, *Legionella pneumophila*, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa* and *Yersinia enterocolitica*.

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Blebs have the advantage of providing outer-membrane proteins in their native conformation and are thus particularly useful for vaccines. Blebs can also be improved for vaccine use by engineering the bacterium so as to modify the expression of one or more molecules at the outer membrane. Thus for example the expression of a desired immunogenic protein at the outer membrane, such as the BASB081 polypeptide, can be introduced or upregulated (e.g. by altering the promoter). Instead or in addition, the expression of outer-membrane molecules which are either not relevant (e.g. unprotective antigens or immunodominant but variable proteins) or detrimental (e.g. toxic molecules such as LPS, or potential inducers of an autoimmune response) can be downregulated. These approaches are discussed in more detail below.

The non-coding flanking regions of the BASB081 gene contain regulatory elements important in the expression of the gene. This regulation takes place both at the transcriptional and translational level. The sequence of these regions, either upstream or downstream of the open reading frame of the gene, can be obtained by DNA sequencing. This sequence information allows the determination of potential regulatory motifs such as the different promoter elements, terminator sequences, inducible sequence elements, repressors, elements responsible for phase variation, the shine-dalgarno sequence, regions with potential secondary structure involved in regulation, as well as other types of regulatory motifs or sequences. This sequence is a further aspect of the invention.

This sequence information allows the modulation of the natural expression of the BASB081 gene. The upregulation of the gene expression may be accomplished by altering the promoter, the shine-dalgarno sequence, potential repressor or operator elements, or any other elements involved. Likewise, downregulation of expression can be achieved by similar types of modification. Alternatively, by changing phase variation sequences, the expression of the gene can be put under phase variation control, or it may be uncoupled from this regulation. In another approach, the expression of the gene can be put under the control of one or more inducible elements allowing regulated expression.

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Examples of such regulation include, but are not limited to, induction by temperature shift, addition of inductor substrates like selected carbohydrates or their derivatives, trace elements, vitamins, co-factors, metal ions, etc.

Such modifications as described above can be introduced by several different means. The modification of sequences involved in gene expression can be carried out *in vivo* by random mutagenesis followed by selection for the desired phenotype. Another approach consists in isolating the region of interest and modifying it by random mutagenesis, or site-directed replacement, insertion or deletion mutagenesis. The modified region can then be reintroduced into the bacterial genome by homologous recombination, and the effect on gene expression can be assessed. In another approach, the sequence knowledge of the region of interest can be used to replace or delete all or part of the natural regulatory sequences. In this case, the regulatory region targeted is isolated and modified so as to contain the regulatory elements from another gene, a combination of regulatory elements from different genes, a synthetic regulatory region, or any other regulatory region, or to delete selected parts of the wild-type regulatory sequences. These modified sequences can then be reintroduced into the bacterium via homologous recombination into the genome. A non-exhaustive list of preferred promoters that could be used for up-regulation of gene expression includes the promoters *porA*, *porB*, *lbpB*, *tbpB*, *p110*, *lst*, *hpuAB* from *N. meningitidis* or *N. gonorrhoeae*; *ompCD*, *copB*, *lbpB*, *ompE*, *UspA1*; *UspA2*; *TbpB* from *M. Catarrhalis*; *p1*, *p2*, *p4*, *p5*, *p6*, *lpD*, *tbpB*, *D15*, *Hia*, *Hmw1*, *Hmw2* from *H. influenzae*.

In one example, the expression of the gene can be modulated by exchanging its promoter with a stronger promoter (through isolating the upstream sequence of the gene, *in vitro* modification of this sequence, and reintroduction into the genome by homologous recombination). Upregulated expression can be obtained in both the bacterium as well as in the outer membrane vesicles shed (or made) from the bacterium.

The vaccine formulation of the invention may also include adjuvant systems for enhancing the immunogenicity of the formulation. Preferably the adjuvant system raises preferentially a TH1 type of response.

An immune response may be broadly distinguished into two extreme categories, being a humoral or cell mediated immune responses (traditionally characterised by antibody and cellular effector mechanisms of protection respectively). These categories of response have been termed TH1-type responses (cell-mediated response), and TH2-type immune responses (humoral response).

Extreme TH1-type immune responses may be characterised by the generation of antigen specific, haplotype restricted cytotoxic T lymphocytes, and natural killer cell responses. In mice TH1-type responses are often characterised by the generation of antibodies of the IgG2a subtype, whilst in the human these correspond to IgG1 type antibodies. TH2-type immune responses are characterised by the generation of a broad range of immunoglobulin isotypes including in mice IgG1, IgA, and IgM.

It can be considered that the driving force behind the development of these two types of immune responses are cytokines. High levels of TH1-type cytokines tend to favour the induction of cell mediated immune responses to the given antigen, whilst high levels of TH2-type cytokines tend to favour the induction of humoral immune responses to the antigen.

The distinction of TH1 and TH2-type immune responses is not absolute. In reality an individual will support an immune response which is described as being predominantly TH1 or predominantly TH2. However, it is often convenient to consider the families of cytokines in terms of that described in murine CD4 +ve T cell clones by Mosmann and Coffman (*Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different*

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patterns of lymphokine secretion lead to different functional properties. Annual Review of Immunology, 7, p145-173). Traditionally, TH1-type responses are associated with the production of the INF- γ and IL-2 cytokines by T-lymphocytes. Other cytokines often directly associated with the induction of TH1-type immune responses are not produced by T-cells, such as IL-12. In contrast, TH2- type responses are associated with the secretion of IL-4, IL-5, IL-6 and IL-13.

It is known that certain vaccine adjuvants are particularly suited to the stimulation of either TH1 or TH2 - type cytokine responses. Traditionally the best indicators of the TH1:TH2 balance of the immune response after a vaccination or infection includes direct measurement of the production of TH1 or TH2 cytokines by T lymphocytes *in vitro* after restimulation with antigen, and/or the measurement of the IgG1:IgG2a ratio of antigen specific antibody responses.

Thus, a TH1-type adjuvant is one which preferentially stimulates isolated T-cell populations to produce high levels of TH1-type cytokines when re-stimulated with antigen *in vitro*, and promotes development of both CD8+ cytotoxic T lymphocytes and antigen specific immunoglobulin responses associated with TH1-type isotype.

Adjuvants which are capable of preferential stimulation of the TH1 cell response are described in International Patent Application No. WO 94/00153 and WO 95/17209.

3 De-O-acylated monophosphoryl lipid A (3D-MPL) is one such adjuvant. This is known from GB 2220211 (Ribi). Chemically it is a mixture of 3 De-O-acylated monophosphoryl lipid A with 4, 5 or 6 acylated chains and is manufactured by Ribi Immunochem, Montana. A preferred form of 3 De-O-acylated monophosphoryl lipid A is disclosed in European Patent 0 689 454 B1 (SmithKline Beecham Biologicals SA).

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Preferably a carrier is also present in the vaccine composition according to the invention. The carrier may be an oil in water emulsion, or an aluminium salt, such as aluminium phosphate or aluminium hydroxide.

A preferred oil-in-water emulsion comprises a metabolisable oil, such as squalene, alpha tocopherol and Tween 80. In a particularly preferred aspect the antigens in the vaccine composition according to the invention are combined with QS21 and 3D-MPL in such an emulsion. Additionally the oil in water emulsion may contain span 85 and/or lecithin and/or tricaprylin.

Typically for human administration QS21 and 3D-MPL will be present in a vaccine in the range of 1µg - 200µg, such as 10-100µg, preferably 10µg - 50µg per dose. Typically the oil in water will comprise from 2 to 10% squalene, from 2 to 10% alpha tocopherol and from 0.3 to 3% tween 80. Preferably the ratio of squalene: alpha tocopherol is equal to or less than 1 as this provides a more stable emulsion. Span 85 may also be present at a level of 1%. In some cases it may be advantageous that the vaccines of the present invention will further contain a stabiliser.

Non-toxic oil in water emulsions preferably contain a non-toxic oil, e.g. squalane or squalene, an emulsifier, e.g. Tween 80, in an aqueous carrier. The aqueous carrier may be, for example, phosphate buffered saline.

A particularly potent adjuvant formulation involving QS21, 3D-MPL and tocopherol in an oil in water emulsion is described in WO 95/17210.

The present invention also provides a polyvalent vaccine composition comprising a vaccine formulation of the invention in combination with other antigens, in particular antigens useful for treating cancers, autoimmune diseases and related conditions. Such a

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polyvalent vaccine composition may include a TH-1 inducing adjuvant as hereinbefore described.

While the invention has been described with reference to certain BASB081 polypeptides and polynucleotides, it is to be understood that this covers fragments of the naturally occurring polypeptides and polynucleotides, and similar polypeptides and polynucleotides with additions, deletions or substitutions which do not substantially affect the immunogenic properties of the recombinant polypeptides or polynucleotides.

Compositions, kits and administration

In a further aspect of the invention there are provided compositions comprising a BASB081 polynucleotide and/or a BASB081 polypeptide for administration to a cell or to a multicellular organism.

The invention also relates to compositions comprising a polynucleotide and/or a polypeptides discussed herein or their agonists or antagonists. The polypeptides and polynucleotides of the invention may be employed in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to an individual. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a polypeptide and/or polynucleotide of the invention and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration. The invention further relates to diagnostic and pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides, polynucleotides and other compounds of the invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

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The pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

In therapy or as a prophylactic, the active agent may be administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably isotonic.

In a further aspect, the present invention provides for pharmaceutical compositions comprising a therapeutically effective amount of a polypeptide and/or polynucleotide, such as the soluble form of a polypeptide and/or polynucleotide of the present invention, agonist or antagonist peptide or small molecule compound, in combination with a pharmaceutically acceptable carrier or excipient. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention. Polypeptides, polynucleotides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

The composition will be adapted to the route of administration, for instance by a systemic or an oral route. Preferred forms of systemic administration include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if a polypeptide or other compounds of the present invention can be formulated in an enteric or an encapsulated formulation, oral administration may also be possible. Administration of these compounds may also be topical and/or localized, in the form of salves, pastes, gels, solutions, powders and the like.

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For administration to mammals, and particularly humans, it is expected that the daily dosage level of the active agent will be from 0.01 mg/kg to 10 mg/kg, typically around 1 mg/kg. The physician in any event will determine the actual dosage which will be most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention.

The dosage range required depends on the choice of peptide, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages, however, are in the range of 0.1-100 µg/kg of subject.

A vaccine composition is conveniently in injectable form. Conventional adjuvants may be employed to enhance the immune response. A suitable unit dose for vaccination is 0.5-5 microgram/kg of antigen, and such dose is preferably administered 1-3 times and with an interval of 1-3 weeks. With the indicated dose range, no adverse toxicological effects will be observed with the compounds of the invention which would preclude their administration to suitable individuals.

Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Sequence Databases, Sequences in a Tangible Medium, and Algorithms

Polynucleotide and polypeptide sequences form a valuable information resource with which to determine their 2- and 3-dimensional structures as well as to identify further sequences of similar homology. These approaches are most easily facilitated by storing the sequence in a computer readable medium and then using the stored data in a known macromolecular structure program or to search a sequence database using well known searching tools, such as the GCG program package.

Also provided by the invention are methods for the analysis of character sequences or strings, particularly genetic sequences or encoded protein sequences. Preferred methods of sequence analysis include, for example, methods of sequence homology analysis, such as identity and similarity analysis, DNA, RNA and protein structure analysis, sequence assembly, cladistic analysis, sequence motif analysis, open reading frame determination, nucleic acid base calling, codon usage analysis, nucleic acid base trimming, and sequencing chromatogram peak analysis.

A computer based method is provided for performing homology identification. This method comprises the steps of: providing a first polynucleotide sequence comprising the sequence of a polynucleotide of the invention in a computer readable medium; and comparing said first polynucleotide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

A computer based method is also provided for performing homology identification, said method comprising the steps of: providing a first polypeptide sequence comprising the sequence of a polypeptide of the invention in a computer readable medium; and comparing said first polypeptide sequence to at least one second polynucleotide or polypeptide sequence to identify homology.

All publications and references, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference in their

entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

DEFINITIONS

"Identity," as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as the case may be, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heine, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Methods to determine identity are designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified in publicly available computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, the GAP program in the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990), and FASTA(Pearson and Lipman Proc. Natl. Acad. Sci. USA 85: 2444-2448 (1988). The BLAST family of programs is publicly available from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894;

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Altschul, S., *et al.*, *J. Mol. Biol.* 215: 403-410 (1990). The well known Smith Waterman algorithm may also be used to determine identity.

Parameters for polypeptide sequence comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Henikoff and Henikoff,
Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

Gap Penalty: 8

Gap Length Penalty: 2

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

Parameters for polynucleotide comparison include the following:

Algorithm: Needleman and Wunsch, *J. Mol Biol.* 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

A preferred meaning for "identity" for polynucleotides and polypeptides, as the case may be, are provided in (1) and (2) below.

(1) Polynucleotide embodiments further include an isolated polynucleotide comprising a polynucleotide sequence having at least a 50, 60, 70, 80, 85, 90, 95, 97 or 100% identity to the reference sequence of SEQ ID NO:1, wherein said polynucleotide sequence may be identical to the reference sequence of SEQ ID NO:1 or may include up to a certain integer number of nucleotide alterations as compared to the reference

sequence, wherein said alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \bullet y),$$

wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, y is 0.50 for 50%, 0.60 for 60%, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, 0.97 for 97% or 1.00 for 100%, and \bullet is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is it may be 100% identical, or it may include up to a certain integer number of nucleic acid alterations as compared to the reference sequence such that the percent identity is less than 100% identity. Such alterations are selected from the group consisting of at least one nucleic acid deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference polynucleotide sequence or anywhere between those terminal positions, interspersed either individually

among the nucleic acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleic acid alterations for a given percent identity is determined by multiplying the total number of nucleic acids in SEQ ID NO:1 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of nucleic acids in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

wherein n_n is the number of nucleic acid alterations, x_n is the total number of nucleic acids in SEQ ID NO:1, y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., \cdot is the symbol for the multiplication operator, and wherein any non-integer product of x_n and y is rounded down to the nearest integer prior to subtracting it from x_n .

(2) Polypeptide embodiments further include an isolated polypeptide comprising a polypeptide having at least a 50,60, 70, 80, 85, 90, 95, 97 or 100% identity to a polypeptide reference sequence of SEQ ID NO:2, wherein said polypeptide sequence may be identical to the reference sequence of SEQ ID NO:2 or may include up to a certain integer number of amino acid alterations as compared to the reference sequence, wherein said alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence, and wherein said number of amino acid alterations is determined by multiplying the total number of amino acids in SEQ ID NO:2 by the integer defining the percent identity divided by 100 and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

"Individual(s)," when used herein with reference to an organism, means a multicellular eukaryote, including, but not limited to a metazoan, a mammal, an ovid, a bovid, a simian, a primate, and a human.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.*, if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living organism is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein. Moreover, a polynucleotide or polypeptide that is introduced into an organism by transformation, genetic manipulation or by any other recombinant method is "isolated" even if it is still present in said organism, which organism may be living or non-living.

"Polynucleotide(s)" generally refers to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA including single and double-stranded regions.

"Variant" refers to a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide, but retains essential properties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, deletions in any combination. A substituted or inserted amino

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acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant; or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Disease(s)" means any disease caused by or related to infection by a bacteria, including, for example, otitis media in infants and children, pneumonia in elderlies, sinusitis, nosocomial infections and invasive diseases, chronic otitis media with hearing loss, fluid accumulation in the middle ear, auditive nerve damage, delayed speech learning, infection of the upper respiratory tract and inflammation of the middle ear.

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EXAMPLES:

The examples below are carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail. The examples are illustrative, but do not limit the invention.

Example 1: DNA sequencing of the BASB081 gene from *Moraxella catarrhalis* strain ATCC 43617.

A: BASB081 in *Moraxella catarrhalis* strain.

The BASB081 gene of SEQ ID NO:1 is from *Moraxella catarrhalis* strain ATCC 43617. The translation of the BASB081 polynucleotide sequence is showed in SEQ ID NO:2.

B: BASB081 in *Moraxella Catarrhalis* strain 43617.

The sequence of the BASB081 gene was confirmed in *Moraxella Catarrhalis* strain ATCC 43617. For this purpose, plasmid DNA (see example 2A) containing the gene region encoding the mature BASB081 from *Moraxella Catarrhalis*. strain ATCC 43617 used as a PCR template. This material was then submitted to Polymerase Chain Reaction DNA amplification using primers *Moraxella catarrhalis* MCD15b-01 (5'-CAT GCC ATG GGT CAA CAA AAT AAC CCT GCA AAC -3') [SEQ ID NO:5] and reverse MCD15b-02 (5'CTA GTC TAG ATT AAA ATG GTG TGC CAA TAA AAA AAT G -3') [SEQ ID NO:6] specific for the BASB081 gene. The PCR amplicon was then submitted to DNA sequencing using the Big Dyes kit (Applied biosystems) and analyzed on a ABI 373/A DNA sequencer in the conditions described by the supplier. As a result, the polynucleotide and deduced polypeptide sequences, referred to as SEQ ID NO:3 and SEQ ID NO:4 respectively, were obtained. These sequences do not comprise the signal sequence as the signal sequence was from the plasmid.

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Using the MegAlign program from the DNASTAR software package, an alignment of the polynucleotide sequences of SEQ ID NO:1 and 3 was performed, and is displayed in Figure 1; a pairwise comparison of identities shows that the two BASB081 polynucleotide gene sequences are 99.9% identical in the region coding for the mature protein. Using the same MegAlign program, an alignment of the polypeptide sequences of SEQ ID NO:2 and 4 was performed, and is displayed in Figure 2; a pairwise comparison of identities shows that the two BASB081 protein sequences are 99.9% identical in the region of the mature protein.

Example 2: Construction of Plasmid to Express Recombinant BASB081

A: Cloning of BASB081.

The *NcoI* and *XbaI* restriction sites (underlined) engineered into the primers *Moraxella catarrhalis* MCD15b-01 (5'- CAT GCC ATG GGT CAA CAA AAT AAC CCT GCA AAC -3') and reverse MCD15b-02 (5'CTA GTC TAG ATT AAA ATG GTG TGC CAA TAA AAA AAT G 3') amplification primers, respectively, permitted directional cloning of a BASB081 PCR product into the commercially available *E. coli* expression plasmid pBADgIII Calmodulin (Invitrogen, USA, ampicillin resistant). This plasmid provides the signal peptide from the bacteriophage fd pIII protein such that a mature BASB081 protein could be targeted to the periplasm of *E. coli*. The BASB081 PCR product was purified from the amplification reaction using Wizard PCR prepTM (Promega) according to the manufacturers instructions. To produce the required *NcoI* and *XbaI* termini necessary for cloning, purified PCR product was sequentially digested to completion with *NcoI* and *XbaI* restriction enzymes as recommended by the manufacturer (Boehringer Mannheim). Digested BASB081 PCR products and pBAD were gel-purified and ligated together using an approximately 5-fold molar excess of the digested fragment to the vector. A standard ~20 µl ligation reaction (~16°C, ~16 hours), using methods well known in the art, was performed using T4 DNA ligase (~2.0 units / reaction, Boehringer Mannheim). An aliquot of the ligation was used to

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SDS/Tris/glycine running buffer. Following electrophoresis, one gel was stained with commassie brilliant blue R250 (BioRad) and then destained to visualize novel BASB081 arabinose-inducible protein(s) (Figure 3).

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Deposited materials

A deposit containing a *Moraxella catarrhalis* Catlin strain has been deposited with the American Type Culture Collection (herein "ATCC") on June 21, 1997 and assigned deposit number 43617. The deposit was described as *Branhamella catarrhalis* (Frosch and Kolle) and is a freeze-dried, 1.5-2.9 kb insert library constructed from *M. catarrhalis* isolate obtained from a transtracheal aspirate of a coal miner with chronic bronchitis. The deposit is described in Antimicrob. Agents Chemother. 21: 506-508 (1982).

The *Moraxella catarrhalis* strain deposit is referred to herein as "the deposited strain" or as "the DNA of the deposited strain."

The deposited strain contains a full length BASB081 gene.

A deposit of the vector pMC-D15 consisting of *Moraxella catarrhalis* DNA inserted in pQE30 has been deposited with the American Type Culture Collection (ATCC) on February 12 1999 and assigned deposit number 207105.

The sequence of the polynucleotides contained in the deposited strain / clone, as well as the amino acid sequence of any polypeptide encoded thereby, are controlling in the event of any conflict with any description of sequences herein.

The deposit of the deposited strains have been made under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for Purposes of Patent Procedure. The deposited strains will be irrevocably and without restriction or condition released to the public upon the issuance of a patent. The deposited strains are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required for enablement, such as that required under 35 U.S.C. §112.

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SEQUENCE INFORMATION

BASB081 Polynucleotide and Polypeptide Sequences

SEQ ID NO:1

Moraxella catarrhalis BASB081 polynucleotide sequence from strain ATCC43617

ATGTCAAAGCCCGTTTGTGTTGCAAATCGCAGTTTATGCCTGTGCGATTGGCGGCTTATTGCGCTTGTATGACATCGCA
AGCATTGGCACAACAAAATAACCTGCAAAATCATCAATCATGTACCCGCTCATGACACCGCCATCAATCAAGCAAAGG
CAGGCAATCCGCTGTTTGTCTAACACCTGAGCAGATACAAGCAGCCTTAATGCTGCTGGACTGAATGCTAAGCCCAA
TCACAAGCTTTGGATGTTGTCAATTTTGTATGATCAATCGCCGATATCTCGTATCGGTGAGCAATCACCCCTTTGGGTTT
GGATATGTCGGTCATCGAAGAAACACACCGCTAAGCTTGGAGGAATTATTGCTCAAGAATCTACTGAGATGGGAATCA
ATCCAAATGATTATATCCAGAATATCAAGGCGAGCAACCTAATAGTGAGGTGGTGTGTACCACCGACATTAGAACCTGAA
AAACCAGGTTTGTATCAAGCGTCTTTATGCACGCTATTTAATGATGGTGTCAATAAGGTGCCTAGGCTTAAGGCAAATT
TTATCAATCATCGCAATCAGGCGAAACCGATGCGATTGGGTCTATCGCATCAAAAAACAGAGCCTTATGCAAAATATCAAAG
CAGCACTTGAAGACATCACCAAGAGTCAGCGATGGATTGAATGGTCTATCCACGCTAAGGCAAATGCTTTGGTG
GCAGCGCTGCTGTCGGTTATTATGATATTGATTATCAATCATAAGAAATAGCATCGGAGAGGTGGATGTCATCATCCA
TGATTAGGTGAACCTGTTTATATTGATTATCGAGCGGTGGAGGTACGAGGTGAAGGTGCTGATGATAAAGCATTACTA
CCGTGGCGGATGAGGTGCCATTGCTGATCGCGATGTCTTTCATCATGGCAAGTACGAAACCAAAAAATCTCATCGAA
AATGCCAGTGCTGAACATGGATATTTGATGGGCGTTGGCTGGATCGTTCAGTTGATGTAATTTTGCAGATAATACCGC
TGATGTCAGCTTAATTTATGATACAGGTACGAGTATCGCTTTGATGAGGTGGTATTTTACCATTGATCCTAAAACCA
ATCAATTGACAACCGATCCAGATAAGCTGCCAGTTAAACGAGAATTACTTGAGCAGTTACTCACCGTTAATCGGGAGAG
GCTTACAATTTACAGGCGGTGCGTGCACTTTCAAATGATTGATTGCCACACGGTATTTAATATGGTGAATACCGAGAT
TGCTTTCCAGAGCGTGAACAGATCCAAACGACCAAGTGAGCTTTGAGCAGTCTTCAAGTAGCCGTAAGCAACGAGC
AAGTTGATGAAAGCACACTTGAACCTGTCATTGAAACCGTTGAGCTAACGGATGGGATATTAATGGATATTTGCGCCATC
GAATTTAGTGATCTAATCTGATTCAAGACAAGCTAAATTTGGTGGCTGCCAAGGCTCGCCATTATATGACATGCGCTGA
TGATAGGGTCTTGCCATCAATCATGATGATGGCGTAAATCGCTCTATTTGGGCAGAATCAGCGATGCCGTATCTGCCG
TTGCACGTGCTATTTTACCTGATGAATCTGAAAATGAGGTAATAGATTGCCCCGAGCGTACCGCATTTGGCTAATCGCAAG
ACCCCTGCTGATGCTATCAAAGTAAAAAGTGCCGCTATATGTCTTTGTGGCGAGTGATAAACCAGAGATGGTCAAAT
TGGTTTGGGCTGGGGATCGGACACAGGTACCCGCTAGTCAAAAAATTGAGCATAATTTGATTAAATCGTGATGGCTATC
AAGCAGGCGCTGAGCTAAGACTGTCTGAGGATAAAAAAGGGTCAAGTTATATGCCACCAACCGCTTAGCCACCCCTTA
AATGATCAGCTAAGAGCAACTTTGGGTTATCAACAAGAAGTTTGTGGTCACTCTACCAATGGTTTGTATTATCCACAG
CACCTTAGAGCATGAGATTAGCCGAGTATTATCCAAATGGTGGCTGGAATCGTACTTATTATTGCGTTATCGTCTTG
ATAAGCTTAAACCAAGCACCCCTGAAACATGGCAGGATTTACCAGTGGATTTTGTCAATGGTAAGCCAAGCCAAGAG
GCGTTATTGGCAGGTGTTGCTGTGCATAAAACGTTGCAGATAATTTGGTTAATCCGATGCGTGGCTATCGTCAGCGATA
TTCTTTAGAGGTTGGCTCAAGCGGTTTGGTATCGGATGCTAATATGGCTATTGCTCGAGCTGGTATTAGTGGCGTGTATA
GTTTGGGGATAATGCTTATGGCAGCAATCGTGCCCATCAGATGACTGGTGGCATAAAGCAGGATACATTGGTGGGAT
AATTTAATCATGTGCCATATCGTTTGCCTTTTGTCTGGTGGCGACCAAGTATTCTGGGATATGCACATGACAGTTT
ATCACCTATATCAGATAAGGTTATCTGACAGGCGGTCAAGTATTGGCGGTTGGTACAGCTGAATATAAATTGAAATTA
TGAAAGATTGCGTTTGGCGGTTTGTGGTATATTGGTAATGCTTATGATAAAGGCTTTACTAATGATACCAAAATTGGT
GCAGGTGTCGGTGTTCGCTGGGCATCACCTGTCGGTCAAGTTCGTGTTGATGTGGCACTGGTGTCAAAGAAGAGGGCAA

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TCCCATTAAGCTGCATTTTATTGGCACACCATTTTAA

SEQ ID NO:2

Moraxella catarrhalis BASB081 polypeptide sequence deduced from the polynucleotide of
SeQ ID NO:1

MSKPVLFANRSFMPVALAAYLPLMTSQALAQNNPANIINHVPADHTAINQAKAGNPPVLLTPEQIQARLNAAGLNAPQ
SQALDVVNFDDQSPISRIGEQSPPLGLDMSVIEETPLSLEELFAQESTEMGINPNDYIPEYQGEQPNSEVVVPPTLEPE
KPGLIKRLYARLFNDGVNKPRLKAKFYQSSQSGETSAIGSSHQKTEPYANIKAALEDITQESAMDNGSIPRLRQTALV
AARAVGYDIDLSIIRNSIGEVVDVIHDLGEPVYIDYRAVEVRGEGADDKAFTTVADEVPLLIGDVFHHGKYETKKNLIE
NASEAHGYFDGRWLDSDVILPDNTADVSLIYDTGTQYRFDEVVFTIDPKTNQLTTPDKLPVKRELLEQLLTVMNGE
AYNLQAVRALSNDLIATRYFNMVNTIEVFPEREQIQNDQVSFEQSSSRTEPAQVDESTLEPVIETVELTDGILMDISPI
EFSASNLIQDKLNLVAAKARHLYDMPDDRVLAINHDDGVNRSILGRISDAVSARAAILPDESENEVIDLPERTALANRK
TPADVYQSKKVPLYVFVASKPRDQIGLGWGS DGTGLVTKFEHNLINRDGYQAGAE LRLSEDKGVKLYATKPLSHPL
NDQLRATLGYYQGEVFGHSTNGFDLSTRLEHEISRSIQNGGWNRTYSLRYRLDKLKTQAPPETWQDLVPDVFVNGKPSQE
ALLAGVAVHKTVDNVLNPMRGYRQYSLVGVSSGLVSDANMAIARAGISGVYSFGDNAYGSNRAHQMTGGIQAQYIWS
NFNHVPYRLRFFAGGDQSIIRGYAHDLSLSPISDKGYLTGGQVLAVGTAENYEFMKDLRLAVFGDIGNAYDKGFTNDTKIG
AGVGVWRWASPVGQVRVDVATGVKEGNPIKLHFFIGTFF

SEQ ID NO:3

Moraxella catarrhalis BASB081 polynucleotide sequence from strain ATCC43617

CAACAAAATAACCTGCAACATCATCAATCATGTACCCGCTCATGACACCGCCATCAATCAAGCAAAGGCAGGCAATCC
GCCTGTTTGTCTAACACCTGAGCAGATACAAGCAGCCTTAATGCTGCTGGACTGAATGCTAAGCCCCAATCACAAGCTT
TGGATGTTGTCAATTTTGATGATCAATCGCCGATATCTCGTATCGGTGAGCAATCACCCCTTTGGGTTTGGATATGTCG
GTCATCGAAGAAACCAACCGCTAAGCTTGAGGAATTATTGCTCAAGAATCTACTGAGATGGGAATCAATCCAAATGA
TTATATTCCAGAAATATCAAGCGAGCAACCTAATAGTGAGGTGGTGTACCACCGACATTAGAACCTGAAAAACAGGTT
TGATCAAGCGTCTTTATGCACGCCTATTAAATGATGGTGTCAATAAGGTGCTTAGGCTTAAGGCAAAATTTATCAATCA
TCGCAATCAGGCGAAACCAAGTCGATTGGGTGATCGCATCAAAAAACAGAGCCTTATGCAAATATCAAAGCAGCACTTGA
AGACATCACCCAGAGTCAGCGATGGATTGAATGGCTCTATCCCACGCCTAAGGCAAACTGCTTTGGTGGCAGCGCGTG
CTGTGCGTTATTATGATATTGATTATCAATCATAAGAAATAGCATCGGAGAGGTGGATGTCATCATCCATGATTAGGT
GAACCTGTTTATATTGATTATCGAGCGGTGAGGTACGAGGTGAAGGTGCTGATGATAAGCATTTACTACCGTGCGGA
TGAGGTGCCATTGCTGATCGCGATGCTTTTCATCACGGCAAGTACGAAACCAAAAAATCTCATCGAAATGCCAGTG
CTGAACATGGATATTTTGATGGCGTGGCTGGATCGTTCAGTTGATGTAATTTTGCCAGATAATACCGCTGATGTCAGC
TTAATTTATGATACAGGTACGCAGTATCGCTTTGATGAGGTGATATTTTTTACCATTGATCCTAAAACCAATCAATTGAC
AACCGATCCAGATAAGCTGCCAGTTAAACGAGAATTACTTGAGCAGTTACTACCGTTAACATGGGAGAGGCTTACAATT
TACAGGCGGTGCGTGCACTTTCAAATGATTTGATTGCCACACGGTATTTTAAATATGGTGAATACCGAGATTGCTTTCCA
GAGCGTGAAACAGATCCAAAACGACCAAGTGAGCTTTGAGCAGTCTTCAAGTAGCCGTACTGAACCAGCACAAAGTTGATGA
AAGCACACTTGAACCTGTCAATTGAAACCGTTGAGCTAACGGATGGGATATTAATGGATATTTGCGCCCATCGAATTAGTG
CATCTAATCTGATTCAAGACAAGCTAAATTTGGTGGCTGCCAAGGCTCGCCATTATATGACATGCCTGATGATAGGGTG
CTTGCCATCAATCATGATGATGGCGTAAATCGCTCTATTTTGGGCAGAAATCAGCGATGCCGTATCTGCGGTGCACGTGC

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TATTTTACCTGATGAATCTGAAAATGAGGTAATAGATTGCCCCGAGCGTACCGCATTGGCTAATCGCAAGACCCCTGCTG
ATGTCTATCAAAGTAAAAAGTGCCGCTATATGTCTTTGTGGCGAGTGATAAACCGAGATGGTCAAATTGGTTTGGGC
TGGGGATCGGACACAGGTACCCGCTAGTCACAAAATTGAGCATAATTGATTAAATCGTGATGGCTATCAAGCAGGCGC
TGAGCTAAGACTGTCTGAGGATAAAAAAGGGTCAAGTTATATGCCACCAACCGCTTAGCCACCCCTCTAAATGATCAGC
TAAGAGCAACTTTGGGTTATCAACAAGAAGTTTTTGGTCACTCTACCAATGGTTTTGATTTATCCACACGACCCCTAGAG
CATGAGATTAGCCGAGTATTATCCAAAATGGTGGCTGGAATCGTACTTATTCATTGCGTTATCGTCTTGATAAGCTTAA
AACCAAGCACCCCTGAAACATGGCAGGATTTACCAGTGGATTTGTCAATGGTAAGCCAAGCCAAGAGGCGTTATTGG
CAGGTGTTGCTGTGCATAAACGGTTGCAGATAAATTGGTTAATCCGATGCGTGGCTATCGTCAGCGATATTCTTTAGAG
GTTGGCTCAAGCGTTTGGTATCGGATGCTAATATGGCTATTGTCGAGCTGGTATTAGTGGCGGTATAGTTTGGGGA
TAATGCTTATGGCAGCAATCGTCCCATCAGATGACTGCTGGCATAACAAGCAGGATACATTGTCGGGATAATTTAATC
ATGTGCCATATCGTTTGGTTTTTTTGTGGTGGCGACCAAGTATTCGTGGATATGCACATGACAGTTTATCACCTATA
TCAGATAAGGGTTATCTGACAGGCGGTCAAGTATTGGCGGTTGGTACAGCTGAATATAATTATGAATTTATGAAAGATT
GCGTTTGGCGGTTTTTGGTGATATTGGTAATGCTTATGATAAAGGCTTTACTAATGATACCAAAATGGTGCAGGTGTCG
GTGTTGCGTGGGCATCACCTGTCGGTCAAGTTCGTGTTGATGTGGCAACTGGTGTCAAAGAAGAGGGCAATCCCATTAAG
CTGCATTTTTTTTATTGGCACACCATTTTAA

SEQ ID NO:4

Moraxella catarrhalis BASB081 polypeptide sequence deduced from the polynucleotide of
SeQ ID NO:3

QQNPNANIINHVPADHTAINQAKAGNPPVLLTPEQIQARLNAAGLNAPQSQALDVVNFDDQSPISRIGEQSPPLGLDMS
VIEETTPLSLEELFAQESTEMGINPNDYIPEYQGEQPNSEVVVPTLEPEKPLIKRLYARLFNDGVNKPRLKAKFYQS
SQSGETSAIGSSHQKTEPYANIKAALEDITQESAMDNGSIPLRLQTALVAARAVGYDIDLSIIRNSIGEVDVIIHDLG
EPVYIDYRAVEVRGEGADDAFTTVADEVPLLIGDVFHHGKYETKKNLIENASAEHGYFDGRWLDERSVDVILPDNTADVS
LIYDTGTQYRFDEVIFFTIDPKTNQLTTPDKLPVKRELLEQLLTVMNGEAYNLQAVRALSNDLIATRYFNMVNTEIVFP
EREQIQNDQVSFEQSSSRTEPAQVDESTLEPVIETVELTDGILMDISPIEFSASNLIQDKLNLVAAKARHLYDMPDDR
LAINHDDGVNRSILGRISDAVSAVARAILPDESENEVIDLPERTALANRKTADVYQSKKVPLYVFVADSKPRDQIGLG
WGS DTGTRLVTKFEHNLINRDGYQAGAE LRLSEDKKGVKLYATKPLSHPLNDQLRATLGYYQEVFGHSTNGFDLSTRILE
HEISRSIIQNGGWNRTYSLRYRLDKLKTQAPPETWQDLPVDFVNGKPSQEALLAGVAVHKTVDNLVNPMPRGYRQRYSL
VGSSGLVSDANMAIARAGISGVYSGDNAYGSNRAHQMTGGIAGYIWSNDFNHVPYRLRFFAGGDQSIIRGYAHDLSLPI
SDKGYLTGGQVLAVGTAEYNYBFMKDLRLAVFGDIGNAYDKGPTNDTKIGAGVGVWRWASPVGQVRVDVATGVKEGNPIK
LHFFIGTPF

SEQ ID NO:5

CAT GCC ATG GGT CAA CAA AAT AAC CCT GCA AAC

SEQ ID NO:6

CTA GTC TAG ATT AAA ATG GTG TGC CAA TAA AAA AAT G

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8. An isolated polynucleotide which comprises a nucleotide sequence which has at least 85% identity to that of SEQ ID NO:1, 3 over the entire length of SEQ ID NO:1, 3 respectively; or a nucleotide sequence complementary to said isolated polynucleotide.
9. The isolated polynucleotide as claimed in any one of claims 6 to 8 in which the identity is at least 95% to SEQ ID NO:1, 3.
10. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4.
11. An isolated polynucleotide comprising the polynucleotide of SEQ ID NO:1, SEQ ID NO:3.
12. An isolated polynucleotide comprising a nucleotide sequence encoding the polypeptide of SEQ ID NO:2, SEQ ID NO:4 obtainable by screening an appropriate library under stringent hybridization conditions with a labeled probe having the sequence of SEQ ID NO:1, SEQ ID NO:3 or a fragment thereof.
13. An expression vector or a recombinant live microorganism comprising an isolated polynucleotide according to any one of claims 6 - 12.
14. A host cell comprising the expression vector of claim 13 or a subcellular fraction or a membrane of said host cell expressing an isolated polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4.
15. A process for producing a polypeptide comprising an amino acid sequence that has at least 85% identity to the amino acid sequence selected from the group consisting of: SEQ ID NO:2, SEQ ID NO:4 comprising culturing a host cell of claim 14 under conditions

sufficient for the production of said polypeptide and recovering the polypeptide from the culture medium.

16. A process for expressing a polynucleotide of any one of claims 6 – 12 comprising transforming a host cell with the expression vector comprising at least one of said polynucleotides and culturing said host cell under conditions sufficient for expression of any one of said polynucleotides.

17. A vaccine composition comprising an effective amount of the polypeptide of any one of claims 1 to 5 and a pharmaceutically acceptable carrier.

18. A vaccine composition comprising an effective amount of the polynucleotide of any one of claims 6 to 12 and a pharmaceutically effective carrier.

19. The vaccine composition according to either one of claims 17 or 18 wherein said composition comprises at least one other *Moraxella catarrhalis* antigen.

20. An antibody immunospecific for the polypeptide or immunological fragment as claimed in any one of claims 1 to 5.

21. A method of diagnosing a *Moraxella catarrhalis* infection, comprising identifying a polypeptide as claimed in any one of claims 1 - 5, or an antibody that is immunospecific for said polypeptide, present within a biological sample from an animal suspected of having such an infection.

22. Use of a composition comprising an immunologically effective amount of a polypeptide as claimed in any one of claims 1 – 5 in the preparation of a medicament for use in generating an immune response in an animal.

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24. A therapeutic composition useful in treating humans with *Moraxella catarrhalis* disease comprising at least one antibody directed against the polypeptide of claims 1 – 5 and a suitable pharmaceutical carrier.

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07K 14/21, C12N 15/00, C07K 16/12		A1	(11) International Publication Number: WO 00/52042
			(43) International Publication Date: 8 September 2000 (08.09.00)
(21) International Application Number: PCT/EP00/01468			(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 23 February 2000 (23.02.00)			
(30) Priority Data: 9904559.3 26 February 1999 (26.02.99) GB			
(71) Applicant (for all designated States except US): SMITHKLINE BEECHAM BIOLOGICALS S.A. [BE/BE]; 89, rue de l'Institut, B-1330 Rixensart (BE).			
(72) Inventor; and (75) Inventor/Applicant (for US only): RUELLE, Jean-Louis [BE/BE]; SmithKline Beecham Biologicals s.a., Rue de l'Institut 89, B-1330 Rixensart (BE).			
(74) Agent: PRIVETT, Kathryn Louise; Corporate Intellectual Property, SmithKline Beecham, Two New Horizons Court, Brentford, Middlesex TW8 9EP (GB).			Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: IMMUNOGENIC COMPOUNDS			
(57) Abstract <p>The invention provides BASB081 polypeptides from moraxella catarrhalis and polynucleotides encoding BASB081 polypeptides and methods for producing such polypeptides by recombinant techniques. Also provided are diagnostic, prophylactic and therapeutic uses.</p>			

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Figure 1 : Alignment of the BASB081 polynucleotide sequences.

Identity to SeqID No:1 is indicated by a dot. Gap is indicated by a dash.

	* 20 *	
Seqid1 :	ATGTCAAAGCCCGTTTTGTTTGCAAATCGC	: 30
Seqid3 :	-----	: -
	40 * 60	
Seqid1 :	AGTTTTATGCCTGTCGCATTGGCGGCTTAT	: 60
Seqid3 :	-----	: -
	* 80 *	
Seqid1 :	TTGCCTTTGATGACATCGCAAGCATTGGCA	: 90
Seqid3 :	-----	: -
	100 * 120	
Seqid1 :	CAACAAAATAACCCTGCAAACATCATCAAT	: 120
Seqid3 :	: 30
	* 140 *	
Seqid1 :	CATGTACCCGCTCATGACACCGCCATCAAT	: 150
Seqid3 :	: 60
	160 * 180	
Seqid1 :	CAAGCAAAGGCAGGCAATCCGCCTGTTTTG	: 180
Seqid3 :	: 90

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400 * 420
Seqid1 : ATGGGAATCAATCCAAATGATTATATTCCA : 420
Seqid3 : : 330

* 440 *
Seqid1 : GAATATCAAGGCGAGCAACCTAATAGTGAG : 450
Seqid3 : : 360

460 * 480
Seqid1 : GTGGTTGTACCACCGACATTAGAACCTGAA : 480
Seqid3 : : 390

* 500 *
Seqid1 : AAACCAGGTTTGATCAAGCGTCTTTATGCA : 510
Seqid3 : : 420

520 * 540
Seqid1 : CGCCTATTTAATGATGGTGTCAATAAGGTG : 540
Seqid3 : : 450

* 560 *
Seqid1 : CCTAGGCTTAAGGCAAAATTTTATCAATCA : 570
Seqid3 : : 480

580 * 600
Seqid1 : TCGCAATCAGGCGAAACCAGTGCGATTGGG : 600
Seqid3 : : 510

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* 620 *

Seqid1 : TCATCGCATCAAAAAACAGAGCCTTATGCA : 630

Seqid3 : : 540

640 * 660

Seqid1 : AATATCAAAGCAGCACTTGAAGACATCACC : 660

Seqid3 : : 570

* 680 *

Seqid1 : CAAGAGTCAGCGATGGATTTGAATGGCTCT : 690

Seqid3 : : 600

700 * 720

Seqid1 : ATCCCACGCCTAAGGCAAACCTGCTTTGGTG : 720

Seqid3 : : 630

* 740 *

Seqid1 : GCAGCGCGTGCTGTCGGTTATTATGATATT : 750

Seqid3 : : 660

760 * 780

Seqid1 : GATTTATCAATCATAAGAAATAGCATCGGA : 780

Seqid3 : : 690

* 800 *

Seqid1 : GAGGTGGATGTCATCATCCATGATTTAGGT : 810

Seqid3 : : 720

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820 * 840
 Seqid1 : GAACCTGTTTATATTGATTATCGAGCGGTG : 840
 Seqid3 : : 750

* 860 *
 Seqid1 : GAGGTACGAGGTGAAGGTGCTGATGATAAA : 870
 Seqid3 : : 780

880 * 900
 Seqid1 : GCATTTACTACCGTGGCGGATGAGGTGCCA : 900
 Seqid3 : : 810

* 920 *
 Seqid1 : TTGCTGATCGGCGATGTCTTTCATCATGGC : 930
 Seqid3 :C... : 840

940 * 960
 Seqid1 : AAGTACGAAACCAAAAAAATCTCATCGAA : 960
 Seqid3 : : 870

* 980 *
 Seqid1 : AATGCCAGTGCTGAACATGGATATTTTGAT : 990
 Seqid3 : : 900

1000 * 1020
 Seqid1 : GGGCGTTGGCTGGATCGTTTCAGTTGATGTA : 1020
 Seqid3 : : 930

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* 1040 *

Seqid1 : ATTTTGCCAGATAATACCGCTGATGTCAGC : 1050

Seqid3 : : 960

1060 * 1080

Seqid1 : TTAATTTATGATACAGGTACGCAGTATCGC : 1080

Seqid3 : : 990

* 1100 *

Seqid1 : TTTGATGAGGTGGTATTTTTTACCATTGAT : 1110

Seqid3 :A..... : 1020

1120 * 1140

Seqid1 : CCTAAAACCAATCAATTGACAACCGATCCA : 1140

Seqid3 : : 1050

* 1160 *

Seqid1 : GATAAGCTGCCAGTTAAACGAGAATTACTT : 1170

Seqid3 : : 1080

1180 * 1200

Seqid1 : GAGCAGTTACTCACCGTTAACATGGGAGAG : 1200

Seqid3 : : 1110

* 1220 *

Seqid1 : GCTTACAATTTACAGGCGGTGCGTGCACTT : 1230

Seqid3 : : 1140

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1240 * 1260
Seqid1 : TCAAATGATTTGATTGCCACACGGTATTTT : 1260
Seqid3 : : 1170

* 1280 *
Seqid1 : AATATGGTGAATACCGAGATTGTCTTTCCA : 1290
Seqid3 : : 1200

1300 * 1320
Seqid1 : GAGCGTGAACAGATCCAAAACGACCAAGTG : 1320
Seqid3 : : 1230

* 1340 *
Seqid1 : AGCTTTGAGCAGTCTTCAAGTAGCCGTACT : 1350
Seqid3 : : 1260

1360 * 1380
Seqid1 : GAACCAGCACAAGTTGATGAAAGCACACTT : 1380
Seqid3 : : 1290

* 1400 *
Seqid1 : GAACCTGTCATTGAAACCGTTGAGCTAACG : 1410
Seqid3 : : 1320

1420 * 1440
Seqid1 : GATGGGATATTAATGGATATTTGCCCCATC : 1440
Seqid3 : : 1350

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* 1460 *

Seqid1 : GAATTTAGTGCATCTAATCTGATTCAAGAC : 1470

Seqid3 : : 1380

1480 * 1500

Seqid1 : AAGCTAAATTTGGTGGCTGCCAAGGCTCGC : 1500

Seqid3 : : 1410

* 1520 *

Seqid1 : CATTTATATGACATGCCTGATGATAGGGTG : 1530

Seqid3 : : 1440

1540 * 1560

Seqid1 : CTTGCCATCAATCATGATGATGGCGTAAAT : 1560

Seqid3 : : 1470

* 1580 *

Seqid1 : CGCTCTATTTTGGGCAGAATCAGCGATGCC : 1590

Seqid3 : : 1500

1600 * 1620

Seqid1 : GTATCTGCCGTTGCACGTGCTATTTTACCT : 1620

Seqid3 : : 1530

* 1640 *

Seqid1 : GATGAATCTGAAAATGAGGTAATAGATTTG : 1650

Seqid3 : : 1560

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1660 * 1680
Seqid1 : CCCGAGCGTACCGCATTGGCTAATCGCAAG : 1680
Seqid3 : : 1590

* 1700 *
Seqid1 : ACCCCTGCTGATGTCTATCAAAGTAAAAA : 1710
Seqid3 : : 1620

1720 * 1740
Seqid1 : GTGCCGCTATATGTCTTTGTGGCGAGTGAT : 1740
Seqid3 : : 1650

* 1760 *
Seqid1 : AAACCACGAGATGGTCAAATTGGTTTGGGC : 1770
Seqid3 :C..... : 1680

1780 * 1800
Seqid1 : TGGGGATCGGACACAGGTACCCGCCTAGTC : 1800
Seqid3 : : 1710

* 1820 *
Seqid1 : ACAAAATTTGAGCATAATTTGATTAATCGT : 1830
Seqid3 : : 1740

1840 * 1860
Seqid1 : GATGGCTATCAAGCAGGCGCTGAGCTAAGA : 1860
Seqid3 : : 1770

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* 1880 *

Seqid1 : CTGTCTGAGGATAAAAAAGGGGTCAAGTTA : 1890

Seqid3 : : 1800

1900 * 1920

Seqid1 : TATGCCACCAAACCGCTTAGCCACCCTCTA : 1920

Seqid3 : : 1830

* 1940 *

Seqid1 : AATGATCAGCTAAGAGCAACTTTGGGTTAT : 1950

Seqid3 : : 1860

1960 * 1980

Seqid1 : CAACAAGAAGTTTTTGGTCACTCTACCAAT : 1980

Seqid3 : : 1890

* 2000 *

Seqid1 : GGTTTTGATTTATCCACACGCACCCTAGAG : 2010

Seqid3 : : 1920

2020 * 2040

Seqid1 : CATGAGATTAGCCGCAGTATTATCCAAAAT : 2040

Seqid3 : : 1950

* 2060 *

Seqid1 : GGTGGCTGGAATCGTACTTATTCATTGCGT : 2070

Seqid3 : : 1980

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2080 * 2100
Seqid1 : TATCGTCTTGATAAGCTTAAAACCCAAGCA : 2100
Seqid3 : : 2010

* 2120 *
Seqid1 : CCCCTGAAACATGGCAGGATTTACCAAGTG : 2130
Seqid3 : : 2040

2140 * 2160
Seqid1 : GATTTTGTCAATGGTAAGCCAAGCCAAGAG : 2160
Seqid3 : : 2070

* 2180 *
Seqid1 : GCGTTATTGGCAGGTGTTGCTGTGCATAAA : 2190
Seqid3 : : 2100

2200 * 2220
Seqid1 : ACGGTTGCAGATAATTTGGTTAATCCGATG : 2220
Seqid3 : : 2130

* 2240 *
Seqid1 : CGTGGCTATCGTCAGCGATATTCTTTAGAG : 2250
Seqid3 : : 2160

2260 * 2280
Seqid1 : GTTGGCTCAAGCGGTTTGGTATCGGATGCT : 2280
Seqid3 : : 2190

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* 2300 *

Seqid1 : AATATGGCTATTGCTCGAGCTGGTATTAGT : 2310

Seqid3 : : 2220

2320 * 2340

Seqid1 : GGCGTGTATAGTTTTGGGGATAATGCTTAT : 2340

Seqid3 : : 2250

* 2360 *

Seqid1 : GGCAGCAATCGTGCCCATCAGATGACTGGT : 2370

Seqid3 : : 2280

2380 * 2400

Seqid1 : GGCATACAAGCAGGATACATTTGGTCGGAT : 2400

Seqid3 : : 2310

* 2420 *

Seqid1 : AATTTTAATCATGTGCCATATCGTTTGCCT : 2430

Seqid3 : : 2340

2440 * 2460

Seqid1 : TTTTTTGCTGGTGGCGACCAAAGTATTCGT : 2460

Seqid3 : : 2370

* 2480 *

Seqid1 : GGATATGCACATGACAGTTTATCACCTATA : 2490

Seqid3 : : 2400

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2500 * 2520
Seqid1 : TCAGATAAGGGTTATCTGACAGGCGGTCAA : 2520
Seqid3 : : 2430

* 2540 *
Seqid1 : GTATTGGCGGTTGGTACAGCTGAATATAAT : 2550
Seqid3 : : 2460

2560 * 2580
Seqid1 : TATGAATTTATGAAAGATTTGCGTTTGGCG : 2580
Seqid3 : : 2490

* 2600 *
Seqid1 : GTTTTTGGTGATATTGGTAATGCTTATGAT : 2610
Seqid3 : : 2520

2620 * 2640
Seqid1 : AAAGGCTTTACTAATGATACCAAATTGGT : 2640
Seqid3 : : 2550

* 2660 *
Seqid1 : GCAGGTGTCGGTGTTGCTGGGCATCACCT : 2670
Seqid3 : : 2580

2680 * 2700
Seqid1 : GTCGGTCAAGTTCGTGTTGATGTGGCAACT : 2700
Seqid3 : : 2610

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```

                *           2720           *
Seqid1  : GGTGTCAAAGAAGAGGGCAATCCCATTAAG : 2730
Seqid3  : ..... : 2640
    
```

```

                2740           *           2760
Seqid1  : CTGCATTTTTTTTATTGGCACACCATTTTAA : 2760
Seqid3  : ..... : 2670
    
```

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Figure 2 : Alignment of the BASB081 polypeptide sequences.**Identity to SeqID No:2 is indicated by a dot. Gap is indicated by a dash.**

```

                *           20           *
Seqid2 : MSKPVL FANRSFMPVALAAYLPLMTSQALA : 30
Seqid4 : ----- : -

                40           *           60
Seqid2 : QQNNPANI INHVP AHD TAINQAKAGNPPVL : 60
Seqid4 : ..... : 30

                *           80           *
Seqid2 : LTPEQIQARLNAAGLNAKPQSQALDVVNFD : 90
Seqid4 : ..... : 60

                100           *           120
Seqid2 : DQSPISRIGE QSPPLGLDMSVIEETPLSL : 120
Seqid4 : ..... : 90

                *           140           *
Seqid2 : EELFAQESTEMGINPNDYIPEYQGEQPNSE : 150
Seqid4 : ..... : 120

                160           *           180
Seqid2 : VVVPPTLEPEKPGLIKRLYARLFNDGVNKV : 180
Seqid4 : ..... : 150
```

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* 200 *

Seqid2 : PRLKAKFYQSSQSGETSAIGSSHQKTEPYA : 210

Seqid4 : : 180

220 * 240

Seqid2 : NIKAALEDITQESAMDNLNGSIPRLRQTALV : 240

Seqid4 : : 210

* 260 *

Seqid2 : AARAVGYDIDLSIIRNSIGEVDVIIHDLG : 270

Seqid4 : : 240

280 * 300

Seqid2 : EPVYIDYRAVEVRGEGADDKAFTTVADEVP : 300

Seqid4 : : 270

* 320 *

Seqid2 : LLIGDVFHHGKYETKKNLINASAHEGYFD : 330

Seqid4 : : 300

340 * 360

Seqid2 : GRWLDRSVDVILPDNTADVSLIYDTGTQYR : 360

Seqid4 : : 330

* 380 *

Seqid2 : FDEVVFFTIDPKTNQLTTDPDKLPVKRELL : 390

Seqid4 : : 360

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400 * 420
Seqid2 : EQLLTVNMGEAYNLQAVRALSNDLIATRYF : 420
Seqid4 : : 390

* 440 *
Seqid2 : NMVNTEIVFPEREQIQNDQVSFEQSSSSRT : 450
Seqid4 : : 420

460 * 480
Seqid2 : EPAQVDESTLEPVIETVELTDGILMDISPI : 480
Seqid4 : : 450

* 500 *
Seqid2 : EFSASNLIQDKLNLVAAKARHLYDMPDDR : 510
Seqid4 : : 480

520 * 540
Seqid2 : LAINHDDGVNRSILGRISDAVSAVARAILP : 540
Seqid4 : : 510

* 560 *
Seqid2 : DESENEVIDLPERTALANRKTPADVYQSKK : 570
Seqid4 : : 540

580 * 600
Seqid2 : VPLYVVFVASKPRDGQIGLGWGS DTGTRLV : 600
Seqid4 : : 570

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* 620 *

Seqid2 : TKFEHNLINRDGYQAGAE LRLSEDKKGVKL : 630

Seqid4 : : 600

640 * 660

Seqid2 : YATKPLSHPLNDQLRATLGYQQEVFGHSTN : 660

Seqid4 : : 630

* 680 *

Seqid2 : GFDLSTRTLEHEISRSIIQNGGWNRTYSLR : 690

Seqid4 : : 660

700 * 720

Seqid2 : YRLDKLKTQAPPETWQDLPVDFVNGKPSQE : 720

Seqid4 : : 690

* 740 *

Seqid2 : ALLAGVAVHKT VADNLVNP MRGYRQ RYSLE : 750

Seqid4 : : 720

760 * 780

Seqid2 : VGSSGLVSDANMAIARAGISGVYSFGDNAY : 780

Seqid4 : : 750

* 800 *

Seqid2 : GSNRAHQMTGGIQAGYIWSDNFNHVPYRLR : 810

Seqid4 : : 780

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820 * 840
Seqid2 : FFAGGDQSIRGYAHDLSLSPISDKGYLTGGQ : 840
Seqid4 : : 810

* 860 *
Seqid2 : VLAVGTAEYNYEFMKDLRLAVFGDIGNAYD : 870
Seqid4 : : 840

880 * 900
Seqid2 : KGFTNDTKIGAGVGVRWASPVGQVRVDVAT : 900
Seqid4 : : 870

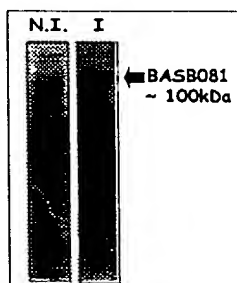
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Seqid2 : GVKEEGNPIKLFHFFIGTPF : 919
Seqid4 :F : 889

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Figure 3. SDS-PAGE analysis of BASB081 expression in non induced (N.I.) or induced (I) *Escherichia coli* Top10 cells.



Docket No.: BM45378

PCT/EP00/01468

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

IMMUNOGENIC COMPOUNDS the specification of which (check one)

☐ is attached hereto.

☒ was filed on 23 February 2000 as Serial No. PCT/EP00/01468
and was amended on (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability as defined in Title 37, Code of Federal Regulations, Section 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) or Section 365(b) of any foreign application(s) for patent or inventor's certificate, or Section 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or Inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Number	Country	Filing Date	Priority Claimed
9904559.3	Great Britain	26 February 1999	Yes

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below.

Application Number Filing Date

I hereby claim the benefit under Title 35, United States Code, Section 120 of any United States application(s) or Section 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each

1995-11-16 10:12:30

of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, Section 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

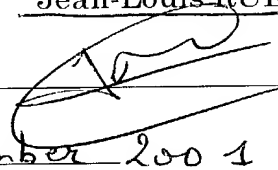
Serial No.	Filing Date	Status
------------	-------------	--------

I hereby appoint the practitioners associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith, and direct that all correspondence be addressed to that Customer Number:

Customer Number 25,308

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

1-00 Full Name of Inventor: Jean-Louis RUELLE

Inventor's Signature: 

Date: 6 December 2001

Residence: Rixensart, Belgium BEX

Citizenship: Belgian

Post Office Address: SmithKline Beecham Corporation
Corporate Intellectual Property - UW2220
P.O. Box 1539
King of Prussia, Pennsylvania 19406-0939

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SEQUENCE LISTING

<110> SmithKline Beecham Biologicals S.A.

<120> Novel compounds

<130> BM45378

<160> 6

<170> FastSEQ for Windows Version 3.0

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<212> DNA

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 Glu Phe Ser Ala Ser Asn Leu Ile Gln Asp Lys Leu Asn Leu Val Ala
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